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EPILEPTIC BRAIN DAMAGE

Histopathological studies on rats with
experimental status epilepticus

Lund 1982



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EPILEPTIC BRAIN DAMAGE - HISTOPATHOLOGICAL STUDIES ON RATS WITH
EXPERIMENTAL STATUS EPILEPTICUS

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II Söderfeldt, B., Blennow, G., Kalimo, H., Olsson, Y., Siesjö, B.K.: Influence of systemic factors on experimental epileptic brain injury. Structural changes accompanying bicuculline-induced seizures in rats following manipulations of tissue oxygenation or α -tocopherol levels. Acc. for publication <u>Acta Neuropath.(Berl)</u>	43

- III Söderfeldt, B., Kalimo, H., Olsson, Y., Siesjö, B.K.:
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- IV Atillo, A., Söderfeldt, B., Kalimo, H., Olsson, Y., Siesjö, B.K.:
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A. BACKGROUND OF PRESENT STUDY

Epilepsy, a disease characterized by recurrent, paroxysmal and synchronous discharges of nerve cells, is a common neurological manifestation, with a total prevalence of about 5 per 1000 in Western countries (Neugebauer and Susser 1979). Apart from the suffering inflicted upon the patient, and the social consequences of the disease, the condition derives its importance from the fact that it may lead to brain damage (Lennox 1942). The present thesis concerns the mechanisms whereby prolonged seizure activity may induce structural alterations in nerve and glial cells. It is based on experimental material, and notably on an animal model in which sustained seizures were induced by the chemical convulsants bicuculline and pentylenetetrazol under conditions in which systemic complications such as arterial hypotension, hypoxia, and hyperthermia were avoided. Before describing these results I will review current information on clinical and experimentally induced epileptic brain damage, briefly discuss cerebral metabolic and circulatory accompaniments of the epileptic discharge, and define the objectives of the study.

1. Structural changes in the brains of patients suffering from epilepsy.

From neuropathological studies it has been known for a long time that patients suffering from epilepsy may have varying degrees of brain damage (Willis 1684, Bouchet et Cazauvielh 1825). Subsequent observations on patients with various epileptic disorders have shown that certain brain regions are more vulnerable than other areas. The hippocampal formation is that part that shows the most constant and pronounced abnormalities (Bouchet and Cazauvielh 1825, Norman 1964, Margerison and Corsellis 1966, Corsellis and Meldrum 1976).

The structural abnormalities in patients with severe epilepsy as seen by light microscopy (LM) are loss of neurons and proliferation of astrocytes which occasionally may result in atrophy and glial scarring. Most often the lesions are more pronounced on one of the sides and within the hippocampal formation there are well-known differences in vulnerability between the various subregions.

Another vulnerable part of the brain is the cerebellum which is damaged in 25-50 percent of all neuropathologically examined patients with epilepsy (Spielmeyer 1927, Margerison and Corsellis 1966). Also in this area there is loss of neurons, particularly the Purkinje cells, and astrocytic gliosis, i.e. proliferation of the so-called Bergman glial cells. Among other regions, the cerebral cortex and the thalamus are also affected in many patients (Scholz 1957, Pfeiffer 1963). Loss of neurons and gliosis often have a laminar distribution but any of the areas of the neocortex may be involved. The distribution of the cerebral and cerebellar lesions in patients with long-lasting severe epilepsy, or in those dying after prolonged status epilepticus, is very similar to that which may be seen in transient, global hypoxia/ischemia (see Spielmeyer 1927, Corsellis and Meldrum 1976). However, the origin of the epileptic lesions is obscure. Since brain damage has been found to exist also in patients with cryptogenic epilepsy (Sommer 1880), they were by many authors considered as a result of the epileptic fits, and particularly of the hypoxic complications occurring during prolonged seizures (Spielmeyer 1927, Scholz 1959, Norman 1964). However, others have maintained that the observed lesions, particularly those affecting the hippocampal formation, are the cause of the epileptic disease (see Margerison and Corsellis 1966, Corsellis and Meldrum 1976). For instance, the hippocampal sclerosis is by some authors regarded to be an effect of birth injury (Earle et al. 1953, Veith 1970). It has also been suggested that, even if birth injury is not present, patients may develop lesions as a result of head injury or cerebral infarctions later in life (Gastaut et al. 1959). Additional results support the hypothesis that brain damage in epileptic patients is not a result of the epileptic discharge per se. Thus, some patients with clinically severe epilepsy may by light microscopy have no detectable brain damage, suggesting that repeated seizure discharge does not necessarily induce gross cellular injury (Margerison and Corsellis 1966).

For obvious reasons, clinical neuropathological material cannot give a clear answer to questions regarding the cause and effects of epilepsy, and it is impossible to differentiate lesions which may be directly related to the fits (primary lesions) from those which are composite in origin i.e. influenced by systemic factors such as hypoxia, hypotension etc. Such important questions are better

suited for experimental analysis in which one can have a good control of various physiological parameters during and after a period of seizures.

2. Structural changes observed in experimentally induced seizures.

To further study the pathogenesis of epileptic brain damage, animal experiments with pharmacologically or electrically induced seizures have been carried out repeatedly. The earliest experiments of this type were done without control of physiological parameters during the seizures (see below). Later on many experiments were conducted under more controlled conditions. In these a variety of substances, as well as electroshock stimuli, were used to induced seizures. However, the results are contradictory. Some authors have found no or minimal lesions even after prolonged seizures (Purpura et al. 1960, Schwartz 1970, Brown et al. 1980, Mouritzen-Dam et al. 1981) whereas others have found relatively pronounced changes (Meldrum et al. 1973, see also Harris 1964, Ben-Ari et al. 1981, Lothman and Collins 1981). Further, the descriptions of the morphological abnormalities are incongruent. Brierley and associates (Meldrum and Brierley 1973, Meldrum et al. 1973, 1974) have in a series of experimental studies emphasized the similarities between epileptic and ischemic brain damage as seen by light microscopy. In these studies there was no electron microscopical analysis of the material. Instead, in the few electron microscopical studies that have been performed after experimentally induced seizures the nerve cell lesions differed from those described in hypoxia-ischemia (Harris 1964, Luse et al 1964, De Robertis et al 1969). Whereas these authors emphasized swelling of glial elements and of endoplasmic reticulum in nerve cells, the lesions caused by hypoxia-ischemia involve marked swelling of mitochondria (Brown and Brierley 1972).

It seems warranted to briefly consider the results obtained with different convulsive agents. When convulsive treatment with pen-tylenetetrazol was introduced in clinical practice this substance was tested on animals (Liebert and Weil 1939, Stecker et al. 1939). Another substance used in early experiments was a seizure-provoking NaCl₃-bleached flour (Silver 1949, Lewy 1950). The neuropathological analysis from these studies gave evidence of rather pronounced changes with neuronal loss and glial reactions, especially in cortex, hippo-

campus and cerebellum. The results are hard to evaluate because physiological parameters were not controlled during the seizures. In later experiments, under more controlled conditions, a variety of different methods was used to induce seizures.

Methionine sulfoximine (MSO) is the toxic substance produced when flour is bleached with nitrogen trichloride. MSO is an analogue and competitive antimetabolite of methionine. Studies by Harris (1964) showed that rabbits and dogs experiencing seizures from this substance had vacuolated foci in the cortex. The neuropil was studded with small and large clear spaces. By electron microscopy the vacuolated foci were found to be swollen cytoplasmic processes of glial cells. De Robertis et al (1967) made an electronmicroscopical study on rats subjected to MSO seizures. They found swelling of nerve endings and loss of synaptic vesicles.

Another substance used is methoxypyridoxine, an antimetabolite of pyridoxine. The results are contradictory. Purpura and Gonzalez-Montaguado (1960) found marked histological changes in the hippocampus of cats perfusion-fixed during seizures that had lasted for 2 to 6 h. On the other hand, Schwartz et al (1970) made a similar study on cats (seizure duration up to 2 h) and found no changes in the hippocampus.

Strychnine was also tested in the above mentioned study by Purpura and Gonzales-Montaguado (1960) but, even after prolonged seizure periods, they found no changes in the hippocampus. Luse et al (1964), on the other hand, noted changes in the oligodendroglial cells in rabbits subjected to strychnine-seizures.

During the last decade, seizures induced by kainic acid, bicuculline and pentylenetetrazol have been extensively studied both regarding the effects on cerebral metabolism and circulation (see below) and regarding morphological changes. Kainic acid, an excitatory substance which induces limbic seizures, seems to have special neurotoxic effects (Foster and Roberts 1980, Lothman and Collins 1981, Ben-Ari et al. 1981). Pronounced neuronal changes, especially in the hippocampal formation, in the cerebellum, and the thalamus have been described. The plant alkaloid bicuculline acts by blocking GABA-mediated inhibition and also by direct non-synaptic action (Heyer et al 1981). When injected intravenously, bicuculline gives a long-lasting status epilepticus in experimental animals. Meldrum, Brierley

and co-workers have studied the effects of bicuculline-induced status epilepticus on baboons and in animals with manifest seizure activity, and a variety of systemic complications (Meldrum and Brierley 1973). Pronounced structural changes were found in the neocortex, cerebellum, thalamus and hippocampus. Light microscopically the neuronal changes were described as "ischemic nerve cell changes" i.e. the same type of alteration that Brierley and co-workers had found after ischemia-hypoxia (Brierley 1971). The progression of nerve cell injury was described in terms of three transitional stages : (1) microvaculation, (2) ischemic nerve cell changes, and (3) ischemic nerve cell changes with incrustations. In hypoxia-ischemia electronmicroscopical studies showed that the vacuoles represented swollen mitochondria with varying disorganization of the cristae (McGee-Russel et al 1970, Brown and Brierley 1972). By analogy, the authors assumed that microvacuoles observed in status epilepticus had a corresponding fine structural counterpart.

In another study on baboons, which were curarized and artificially ventilated, no changes were found in the cerebellum and the changes in neocortex and hippocampus were less pronounced than in nonparalysed baboons (Meldrum et al 1973). However, the neuronal alterations observed appeared to be of the same type as previously described.

Blennow et al (1978) studied bicuculline-induced status epilepticus in rats, and deliberately varied arterial PO_2 , blood pressure, and body temperature. Also in this study, neuronal changes of the "ischemic nerve cell" type were found in cortex, thalamus and hippocampus. The surprising result was, however, that mild hypotension or hypoxia during the seizure period seemed to diminish the degree of damage. It was proposed by the authors that neuronal damage in animals with unrestricted cerebral oxygen availability is due to oxidative mechanisms, e.g. due to free radical formation (cf. Siesjö 1981).

Another study on bicuculline induced status epilepticus was reported by Brown et al (1981). Contradictory to what earlier had been described they found no changes in the cat hippocampus even after prolonged seizures. However, their methodology was different in that the cats were sacrificed first after a recovery period of 1-5 hrs.

Pentylenetetrazol is, like bicuculline, classified as a GABA antagonist but its action seems to be at a different site than at the GABA-receptor itself (Marangor et al 1979, Simmons 1980). Apart from the above mentioned studies from the thirties, some more recent studies are reported. Two electron-microscopical studies with pentylenetetrazol (Luse et al 1964, De Robertis et al 1969) have both given evidence of changes in glial cells and neuropil. Ben-Ari et al (1981) have studied pentylenetetrazol induced seizures on unanesthetized, unrestrained rats. The animals showed degenerative changes mainly in the cerebellum, but after longer survival periods also in the hippocampus, amygdala, cortex and thalamus. In the cat, though, seizures induced by pentylenetetrazol were found not to cause cell damage in the hippocampus, although seizure discharge lasted for 3 to 5 h (Purpura et Gonzales-Montaguado 1960). We note that, in this model, damage to hippocampal pyramidal cells was observed during seizures induced by methoxypyridoxine, but not by strychnine and pentylenetetrazol (see above).

Apart from the pharmacologically induced epileptic fits, electro-shock seizures have also been studied. Some slight morphological alterations in cats sacrificed in the immediately post-ictal period were described by Mölbert et al (1967). However, subsequent investigators have not found signs of irreversible cell damage even if animals are exposed to more than 100 shocks (Brennan et al 1972, Mouritzen-Dam et al 1980).

In conclusion, many neuropathological studies on experimental animals have been performed, but it still remains uncertain if epileptic seizures per se give rise to persistent damage in the brain in the absence of systemic complications. It should also be recalled that many substances, e.g. kainic acid, may have a direct neurotoxic effect, apart from their ability to induce seizures. There is also another factor that makes it hard to evaluate many studies, i.e. the fixation technique. Artefacts are easily produced when brain tissue is processed for microscopy (Cammermeyer 1978). In many studies on epilepsy the brains have been fixed by immersion, which easily gives artefacts. Also when perfusion fixation has been performed, the fixative used or other parts of the morphological techniques could have been improper, and given rise to artefacts.

3. Status epilepticus as a model for epileptic brain damage

In view of the results presented it would seem that further attempts to elucidate the mechanisms of epileptic brain damage requires that prolonged seizures can be induced on animals, in which physiological variables can be controlled and, if necessary, manipulated. In this respect, bicuculline and pentylenetetrazol appear to offer some advantages since they have no known direct neurotoxic effect, they give rise to sustained seizures, and these have been extensively characterized in terms of cerebral metabolic and circulatory accompaniments. I and my colleagues therefore chose the model of bicuculline-induced seizures to further characterize the morphological changes during and shortly after seizures of 1 and 2 h duration. At the time when I started my experiments there was only one previous morphological report, exclusively based on LM analysis (Blennow et al. 1978). When the results on bicuculline-induced seizures appeared, we felt the need to explore whether the results only applied to that model or if they were valid for other convulsants as well. We therefore undertook a similar analysis on seizures induced by pentylenetetrazol.

In the rat, bicuculline-induced seizures give rise to a 2- to 3-fold increase in cerebral cortical oxygen and glucose consumption (Meldrum and Nilsson 1976, Borgström et al 1976). However, at least during the first 60 min there is a corresponding, or even greater, increase in cerebral blood flow (CBF) suggesting that cerebral oxygenation is upheld (cf. Duffy et al. 1975, Chapman et al. 1977). Between 60 and 120 min, blood flow rates decrease in cerebral cortical and limbic structures in spite of upheld metabolic rates (Ingvar and Siesjö 1982). It is not known if this "mismatch" is pathogenetically important, but it should be noted that it affects structures considered to be "vulnerable" (see below).

Useful background information to the discussion which will follow is a brief account of cerebral metabolic changes during bicuculline-induced seizures. Although seizure onset leads to a small perturbation of cerebral energy state, tissue ATP, ADP, and AMP concentrations subsequently stabilize at values close to normal (Chapman et al 1977, Blennow et al 1979). In other words, sustained seizures do not lead to overt "energy failure". However, a moderate to marked lactic acidosis develops which is exaggerated by induced

hypoxia or hypotension (Blennow et al. 1978). In spite of the maintained energy state, the extracellular K^+ concentration increases and that of Ca^{2+} decreases, signifying cellular release of K^+ and uptake of Ca^{2+} (Astrup et al. 1979, Heinemann 1977). There is also marked accumulation of free fatty acids, particularly of arachidonic acid (Siesjö et al. 1982). Thus, conditions are at hand for adverse cellular reactions, triggered by increased concentrations of Ca^{2+} and arachidonic acid, the latter serving as a precursor for prostaglandins and related substances, some of which have free radical character (see Siesjö 1981). It has been speculated, but not proven, that such alterations give rise to cellular damage.

Cerebral circulatory and metabolic changes during pentylenetetrazol seizures resemble these elicited by bicuculline (Plum et al. 1968, Howse et al 1964, Duffy et al 1975). However, no data exist on seizures of long duration.

4.Objectives of the present study

In this thesis I wish to present experimental data concerning the effects of status epilepticus on the mammalian brain with emphasis on the light- and electronmicroscopical features. The following aspects are the most important ones :

1. To characterize the lesions which might be induced in cerebral cortex and hippocampus during bicuculline-induced status epilepticus in the rat. Topographical distribution as well as the light (LM) and electron microscopical(EM) characteristics of the lesions were examined since such factors may give us a clue about their pathogenesis.
2. To study the effects on structural changes of variation in arterial oxygenation, arterial blood pressure, and brain α -tocopherol level, i.e. an effort to differentiate between primary and composite lesions.
3. To study whether any of the morphological changes can be reversed during the early recovery period after status epilepticus.
4. To find out whether the changes in blood flow, free fatty acids, energy metabolites and morphology occurring during pentylenetetrazol-induced status epilepticus are similar to those in bicuculline-induced status epilepticus. In this way I wanted to find out if the changes detected in the bicuculline-treated rats are restricted to this model of status epilepticus.

B. METHODS

General experimental method

The experiments were carried out on male Wistar rats (Møllegaards Avelslab, Copenhagen) with free access to water and rat pellets prior to the experiments. Anesthesia was induced with 3 per cent halothane; the animals were then tracheotomized, immobilized with d-tubocurarine chloride, and artificially ventilated with nitrous oxide/oxygen (7/3). For continuous blood pressure (BP) recording and for blood sampling the femoral arteries were cannulated. Intravenous injections and donor blood were given via a catheter in one femoral vein.

The electroencephalogram was recorded from gold-plated bolts in the skull bone. Rectal temperature was maintained close to 37° C by external heating. After the operative procedure was completed, the animals were given heparine i.v. and maintained at steady state for 15-20 min after withdrawal of halothane. Before induction of seizures, the blood pressure was reduced from 140-160 mm Hg to 100 mm Hg by bleeding. This was done to diminish the severity of early ictal hypertension.

Two different substances were used to induce seizures; bicuculline and pentylenetetrazol. Bicuculline (Sigma Chemical Corp.) was dissolved in 0.1 N HCl and brought to pH 4-5 and injected i.v. in a dose of 1.2 mg.kg⁻¹ body weight.

Pentylenetetrazol in aq. solution (University Hospital Pharmacy, Lund) was injected i.v. in a dose of 100 mg.kg⁻¹.

After 5 min of seizure activity N₂O in the gas mixture was changed to N₂. Except for the groups where manipulations of oxygenation or blood pressure were studied, ventilation was adjusted to given an O₂-tension above 100 mm Hg and arterial blood pressure was maintained above 100 mm Hg by infusion of heparinized donor blood.

Morphological methods

At the end of the experiments the brain was fixed by vascular perfusion through a metal cannula in the ascending aorta, after a brief rinse with saline. Three different fixatives were used :

- a. Glutaraldehyde 3 % in 0.10 mol.l⁻¹ phosphate buffer, pH 7.4 37° C.
- b. Glutaraldehyde 3 % in 0.15 mol.l⁻¹ phosphate buffer, pH 7.4 37° C.

c. Formaldehyde, glacial acetic acid and absolute methanol (FAM) (David 1955) in ratios 1:1:18, pH 3.4 37° C.

We allowed at least 250 ml of the glutaraldehyde fixatives to pass the vascular bed and about 1500 ml of the FAM fixative. Perfusion pressure was 135 mm Hg and the duration about 20 min. After perfusion the brain was left in situ for at least 45 min; it was then removed and stored in the same fixative until processed for microscopy. Efficiency of fixation was evaluated by absence of blood in vessels. One hemisphere of the brain was coronally sectioned and embedded in plastic (JB-4, Polysciences inc., or EFL-67 Serva Feinbiochemica). From the other hemisphere samples were taken for high resolution LM and EM and for embedding in paraffin. The JB-4 and EFL-67 embedded sections were stained with toluidine blue and the paraffin sections with hematoxylin-eosin and cresyl violet.

The cortical tissue blocks for EM were sectioned coronally into 230 nm slices with a Sorvall TC-2 tissue sectioner. The slices were postfixed in 1 % osmium tetroxide in cacodylate buffer, stained en bloc with 2 % uranyl acetate in maleate buffer, dehydrated in a graded series of ethanol, and embedded in epon. Semithin sections stained with toluidine blue were used for the selection of areas for thin sections, which were double stained with uranyl acetate and lead citrate and examined in an electromicroscope.

Experimental groups and manipulations induced

Paper I This study includes animals with bicuculline-induced status epilepticus for 1 or 2h. All three fixatives were used to evaluate their respective effects and the cerebral cortex only was examined.

Paper II deals with the effects of variation of arterial oxygenation, blood pressure and brain α -tocopherol level on the structural alterations in bicuculline-induced seizures. To that end, one group of animals was made hypotensive (arterial blood pressure 50 or 70 mmHg) by bleeding a few minutes after the induction of the seizures. Another group was made hypoxic (arterial pO₂ 50 mmHg) by lowering oxygen concentration in the insufflated gas mixture. The effects of hyperoxygenation were tested by ventilating one group of animals on 100 per cent O₂. To evaluate the effects of variation in

tissue α -tocopherol levels one group of animals was given vitamin E i.p. prior to the experiments, and another group was fed on vitamin E deficient food after weaning off. As controls served animals subjected to hypoxia or hypotension alone, and animals with vitamin E deficiency without status epilepticus.

In Paper III we studied the reversibility of morphological changes in cerebral cortex in the early recovery period. Seizure activity was arrested by i.v. injections of diazepam after 1 or 2h of EEG seizures. The animals were then allowed to survive 5 min or 2h, respectively, before perfusion fixation.

Paper IV is a study of hippocampal lesions during bicuculline-induced status epilepticus for 1 or 2h. Recovery was studied as well.

Paper V is a study of pentylenetetrazol-induced status epilepticus. The main purpose of the study was to explore if the results obtained with bicuculline-induced seizures were representative for sustained seizures in general. However, since it was not known if the agent used induced similar cerebral metabolic and circulatory changes as bicuculline, separate groups were studied to settle this issue.

C. RESULTS AND COMMENTS

Physiological and EEG changes

Upon initiation of seizures by bicuculline or pentylenetetrazol an initial rise of arterial blood pressure to 170-200 mm Hg was consistently observed. The pressure began to decline 5-15 min after onset of seizures. In animals that were not deliberately made hypotensive MABP was maintained above 100 mm Hg by infusion of donor blood.

When diazepam was injected in the recovery animals, there was a further tendency to hypotension, which was only partly affected by injections of donor blood. In the 2 h epilepsy group mean arterial blood pressure could not be kept at an acceptable level during recovery.

The onset of seizures was in EEG characterized by rapid, irregular spikes, followed by spikes of greater amplitude and rhythmicity. This pattern was later changed into one with bursts of rapid,

irregular spikes, lasting 1-30 sec, separated by periods of EEG flattening of 1-10 sec duration. In the group with moderate arterial hypotension and the group with hypoxia during the seizures there were periods with single spikes or polyspike and wave activity interspersed with bursts of fast spikes.

When diazepam was injected in the recovery animals, the EEG spikes slowly declined and the EEG became isoelectric. After a period of 60 min an EEG activity returned.

1. Morphological changes during bicuculline-induced status epilepticus

In the cerebral cortex marked changes were observed in all rats taken immediately after a seizure period of 1-2 h. The structural alterations observed were nerve cell changes and status spongiosus. Status spongiosus was seen particularly in cortical layer 3 where it usually had a laminar distribution. Similar areas were also to a lower degree present in deeper cortical layers as well, especially in rats subjected to 2 h of seizures. Electron microscopy showed that this pattern was formed by swollen astrocytic processes, often with a perivascular position. They were also commonly located close to the altered neurons in layer 3.

Nerve cell changes. On the basis of the LM appearance, two main types of neuronal alterations could be characterized. The most frequent neuronal abnormality (injury type 1) was in LM characterized by a change in staining quality, giving the neurons a darkstaining appearance. The neurons also had a shrunken cytoplasm and were surrounded by perineuronal vacuoles. This type of injured neuron was by LM further divided into two subpopulations, 1a and 1b. In type 1a neurons, the nuclei could be distinguished from the cytoplasm, but in type 1b, internal structures could not be discriminated from each other. Electron microscopy showed that the darkly staining type 1a neurons had a slight dilatation of endoplasmic reticulum and contained some vacuoles in the cytoplasm. There was little indication that the vacuoles represented ballooned mitochondria. Instead, the mitochondria looked normal. Also the darkly staining type 1b neurons contained some vacuoles but most of them were so small that a mitochondrial origin is unlikely. The ribosomes and the rough endoplasmic reticulum (RER) cisternae were tightly packed. In the nuclei the chromatin was aggregated into large clumps. Often a massive accumulation of trans-

mitter vesicles in axon terminals making synaptic contact with type 1a and 1b neurons was noticed.

The other type of injured neurons (type 2), had normal staining characteristics but had slit-formed cytoplasmic vacuoles. This type of injured neurons was especially common in cortical layer 5. Most slit-formed vacuoles represented widened portions of RER including the nuclear envelope. They also contained small vacuoles, probably dilated Golgi cisternae. Most mitochondria looked normal, and vacuoles with crista-like material rarely could be found. Analysis of the cortex showed that after 1 and 2 h respectively 33 and 44 per cent, respectively, of the neurons showed alterations of type 1a and b resp. 12 per cent type 1b changes. Type 2 alterations were present in about 8 per cent of the neurons.

In the hippocampus, stratum pyramidale from CA1 and CA3 as well as the pyramidal cell area of CA4 showed marked status spongiosus. Nerve cell changes were also present but less severe than those in the cortex. Most neurons had only mild to moderate configurational changes (type 1a change) but some shrunken and darkstaining type 1b neurons were also found. Ultrastructurally, they show the same changes as found in similar cells in the cortex.

In area dentata pyramidal basket neurons of stratum granulosum and pyramidal nerve cells in stratum polymorphe showed severe type 1b changes.

Compared to the parietal cortex the type 2 changes were extremely rare. As in the cortex, the changes were somewhat more pronounced after 2 h of seizure activity than after 1 h.

No discrepancy could be detected between the two different glutaraldehyde buffered fixatives. The FAM fixative gave in our hands a very poor preservation of the tissue even in controls. We therefore found that a meaningful evaluation of the material was impossible. Some further points need to be discussed regarding the eventually artefactual origin of the observed lesions in the glutaraldehyde fixed brains. We have evaluated the efficiency of fixation by macro- and microscopical examination of the brains. They were all firm in consistency and brownish-yellow in color. In light microscopy we found that blood vessels were patent and free of blood constituents. The brains of the controls looked normal both in LM and EM (Peters and Palay 1976).

The neurons of the type 1 category described in our study may show some coarse resemblance to the artefactual dark neurons described by Cammermeyer (1978). A careful analysis shows that there are several differences. The dark neurons described by Cammermeyer are preferentially found in the transitional zone between gray and white matter. The dark neurons in our material mostly occurred in cortical layer. Furthermore, we have not observed the "cork-screw"-like dendrites described by Cammermeyer.

Compared to other studies on experimental epilepsy, we have found changes that are different from those described by Brierley and coworkers. Since the experimental conditions have been very similar we regard that the differences in fixation techniques is the source of much of the discrepancy. Further, as no electron microscopical study has been performed by Brierley and coworkers their conclusion that status epilepticus is associated with swelling of mitochondria is only based on earlier studies on hypoxia-ischemia (McGee-Russel et al 1970, Brown and Brierley 1972). There are some earlier studies that may support our findings on the morphological changes during epileptic seizures. Luse et al (1964) found swelling of neuronal endoplasmic reticulum in strychnine-induced seizures. A similar reaction was noted by Harris (1964). Also the swelling of perivascular and perineuronal glial cells has been described earlier (Harris 1964, Luse et al 1964, DeRobertis et al 1969).

2. Transient and persistent changes in the early recovery period

Cortex. Already 5 min following the arrest of seizure activity, following a seizure period of 1h, most of the astrocytic edema had vanished. The number of type 1 injured neurons was clearly reduced and type 2 injured neurons had almost disappeared.

After 2 h of recovery, following 1 h of status epilepticus, the edema was virtually absent and no type 2 injured neurons were found. The type 1 nerve cell damage now affected less than 2 % of the neuronal population.

When recovery was instituted after 2 h of status epilepticus, an adequate blood pressure could not be obtained. The animals in this group had numerous dark, triangular neurons in the cortex, but these alterations were probably not the result of the seizure activity per se.

Hippocampus. Also in the hippocampus, the edema had disappeared after 2 h of recovery in animals with 1 h of epilepsy. The type 1 a changed neurons had vanished almost entirely, but about 1 per cent condensed and darkstaining type 1 b injured neurons remained. The results then demonstrate that virtually all of the cellular changes observed by LM and EM during status epilepticus of 1-2h are reversible in the immediate recovery period. This model of status epilepticus has, however its shortcomings. Longer recovery periods are not possible to study due to systemic complications. Thus, although we can conclude that the great majority of the structural alterations observed during status epilepticus quickly disappears in the recovery period, no definitive conclusions about the degree of reversibility of the epileptic brain damage can be drawn.

3. Effects of systemic factors and α -tocopherol administration or depletion.

There are reasons to believe that the brain cell injuries seen in patients and many experimental situations have a composite etiology. In a previous paper the provocative finding was presented that hypoxia or hypotension during the seizures diminished the degree of damage (Blennow et al 1978). Therefore, we wanted to find out how and to what extent changes in various systemic factors influenced the outcome of bicuculline-induced seizures of the same type as presented above, using both high resolution LM and EM in the analysis. Our original intention was to find out if hyperoxia could exaggerate the lesions observed. However, since the control hypoxic animals did not show the expected structural alterations, the analysis was extended to include hyperoxia, manipulation of α -tocopherol levels, hypoxia, and hypotension.

The non-epileptic animals exposed to comparable degrees of hypoxia or hypotension showed no or only minimal structural alterations. Neither did the control animals subjected to α -tocopherol depletion show any detectable changes in the cerebral cortex.

In the epileptic animals hyperoxia did not change the quality or extent of the structural alterations observed in normoxic epileptic animals. In the hypoxia group, the extent of cell damage was somewhat larger than in normoxic, epileptic animals. The ultrastructural characteristics were somewhat different. In many type 1a and 1b neurons the mitochondria were enlarged and the cristae ruptured. They also had more pronounced astrocytic changes with swelling of astrocytic nuclei. Similar changes were observed in animals subjected to hypotension of 50 mmHg during the seizures. Animals with hypotension of 70 mmHg showed the same changes as normotensive animals.

Neither administration nor deficiency of vitamin E modified the pattern of alterations.

The results argue against the hypothesis that epileptic brain damage may be "oxidative", e.g. triggered by free radical formation (Blennow et al 1978). Instead they support the clinical suspicion that hypoxia or hypotension during prolonged seizures can increase the extent of brain damage to the brain.

4. Comparison between bicuculline- and pentylenetetrazol-induced seizures

The alterations in EEG activity, in cerebral metabolism and circulation were similar in both the models. The histopathological study on rats with pentylenetetrazol-induced status epilepticus shows that they had pronounced nerve cell changes and astocytic edema in the cortex and hippocampus. The cerebellum appeared light microscopically normal. Nerve cell changes were of the same type as in bicuculline-induced status epilepticus and could then be classified in the same categories (see above). Also the degree of neuronal change was the same as in bicuculline-induced status epilepticus of the same duration. Electron microscopically the neuronal changes were moreover mainly of the same type as in bicuculline-induced seizures, with the exception that mitochondrial lesions appeared more often in the type 1 injured neurons. Thus, this study shows that the described damages are not specific for bicuculline-induced status epilepticus. They show also when seizures are induced by another drug, assumed to cause

dysinhibition by interacting with GABA-ergic mechanisms at another site.

D. CONCLUSIONS

1. Bicuculline- and pentylenetetrazol-induced status epilepticus in the rat give marked acute changes in the cerebral cortex and - to a lesser degree - the hippocampus.

2. The changes consist of nerve cell changes and status spongiosus. The neuronal changes are different from those observed in hypoxia-ischemia and consist of darkstaining neurons with condensation of cytoplasm (type 1) and neurons with expanded slitformed vacuoles of endoplasmic reticulum (type 2).

3. Induced hyperoxia and/or α -tocopherol deficiency do not alter the extent or quality of morphological changes observed. However, if the animals are made moderately hypoxic or hypotensive during the seizures, the changes become more pronounced and mitochondrial damage is observed in the neurons.

4. The edema and most of the neuronal changes are reversible within 2 h if the seizures are stopped by diazepam. A few damaged neurons (about 2 per cent in the cortex) persist and are probably irreversibly damaged.

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Pathogenesis of Brain Lesions Caused by Experimental Epilepsy*

Light- and Electron-microscopic Changes in the Rat Cerebral Cortex Following Bicuculline-induced Status Epilepticus

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Summary. Status epilepticus was induced in rats by the GABA receptor blocking agent, bicuculline, during artificial ventilation and with closely monitored physiologic parameters. After 1 or 2 h of status epilepticus the brains were fixed by perfusion with glutaraldehyde and processed for light and electron microscopy.

In the cerebral cortex two different types of changes were present, i.e., nerve cell injuries and status spongiosus. Type 1 injured neurons, mainly in the areas of most marked sponginess (layer 3), displayed progressive condensation of both karyo- and cytoplasm. In the most advanced stages the nucleus could no longer be distinguished from the cytoplasm in the light microscope, and vacuoles of apparent Golgi cisterna origin appeared in the darkly stained cytoplasm. This type of injured neurons comprised 41 and 56% of the cortical neurons after 1 or 2 h of status epilepticus, respectively.

Seven to 9% of the neurons showed another type of injury (type 2). They were mainly located in the deeper cortical layers, and showed slit-formed cytoplasmic vacuoles chiefly due to swelling of the endoplasmic reticulum including the nuclear envelope.

Marked sponginess of the cortex developed principally in layer 3 and it spread into deeper layers with longer duration of status epilepticus, but the outermost layers retained a compact structure. As judged by electron microscopy, the sponginess resulted mainly from swelling of astrocytes and their processes causing both perivascular and perineuronal vacuolation.

The structural changes observed are considered to be caused by astrocytic and to a lesser extent intraneuronal edema related to the seizure activity.

Although the exact pathogenetic mechanisms are not known, our findings indicate that hypoxia-ischemia is not a major determinant of the tissue damage observed.

Key words: Status epilepticus — Nerve cell injury — Brain edema — Rat cerebral cortex

Introduction

The mechanisms causing cell damage in the brain in epileptic seizures have not yet been defined. In fact, it is still an open question whether morphological changes are caused by the epileptic activity per se or by factors complicating the seizure discharge. Thus, although it has been clearly documented that cell damage, mainly affecting neurons in the cerebral cortex, hippocampus and cerebellum occurs in patients with repeated seizures (Spielmeyer 1927; Scholtz 1959; Norman 1964; Salzman et al. 1978) it has not been possible to exclude complicating factors, such as hypoxia, as contributory causes. Furthermore, some authors have suggested that epileptic lesions lack characteristic light-microscopic (LM) features in that they appear identical to "ischemic cell changes", i.e., neuronal alterations commonly induced by severe hypoxia/ischemia (Spielmeyer 1922; Scholtz 1959; Norman 1964; Meldrum and Brierley 1973).

Since physiologic variables can be monitored and/or controlled in animal experiments, several groups have studied neuropathologic alterations accompanying drug-induced seizures of varying duration. Experiments in baboons have shown that a certain minimal period of seizure activity is needed to induce LM evidence of neuronal damage (Meldrum and Brierley 1973; Meldrum et al. 1973, 1974). Furthermore, they have demonstrated that artificial

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ventilation and adequate oxygenation of arterial blood reduce the extent of brain damage. However, prolonged periods of seizure activity (3 h) nevertheless led to cell changes in selectively vulnerable areas, mainly the neocortex and the hippocampus (Meldrum et al. 1973).

Meldrum and Brierley (1973) and Meldrum et al. (1973) emphasized the similarity between the seizure-induced lesions and "ischemic nerve cell damage". The progression of nerve cell injury was described in terms of three transitional stages: (1) microvacuolation, (2) ischemic cell change, and (3) ischemic cell change with incrustations eventually leading to dissolution and disappearance of the cells (e.g. Meldrum and Brierley 1973). In hypoxia-ischemia electron-microscopic (EM) studies showed that the microvacuoles represented swollen mitochondria with variable disorganization of the cristae (McGee-Russel et al. 1970; Brown and Brierley 1972). By analogy, the authors assumed that microvacuoles observed in status epilepticus had a corresponding fine structural counterpart (Meldrum and Brierley 1973; Meldrum et al. 1973).

There are remarkably few earlier studies which have taken the opportunity of using EM to characterize the brain lesions in status epilepticus, and these studies do not conform to the hypothesis that status epilepticus gives the same type of neuropathologic alteration as in ischemia-hypoxia. Luse et al. (1964) emphasized that in strychnine-induced seizures neuronal changes consisted in widening of the endoplasmic reticulum in the absence of mitochondrial changes. A similar neuronal reaction was also noted by Harris (1964) in experiments with methionine sulfoximine-induced seizures. Another prominent alteration described in the early EM reports was swelling of perivascular and perineuronal glial cells (Harris 1964; Luse et al. 1964; de Robertis et al. 1969).

A recent LM study on brains perfusion-fixed with a formalin-acetic acidmetanol (FAM) mixture showed that when bicuculline-induced status epilepticus is

sustained for 2 h in paralyzed and artificially ventilated rats, unequivocal "ischemic cell damage" occurs in selectively vulnerable areas, mainly the cerebral cortex and hippocampus (Blennow et al. 1978). Since this model, in which the seizures are induced by the GABA receptor blocking agent bicuculline, has been extensively characterized in terms of circulatory and metabolic changes (Meldrum and Nilsson 1976; Chapman et al. 1977) it lends itself to analyses with morphological methods. The present study was undertaken to allow a more detailed characterization of the changes in the cerebral cortex. Specifically, we wanted to study whether or not the lesions observed in the tissue, optimally processed for LM and EM, resemble those described in certain forms of hypoxia-ischemia.

Material and Methods

Animals and Model

The experiments were carried out on male Wistar rats (290–350 g) with free access to water and rat pellets prior to the experiments. The number of rats in the experimental groups is presented in Table 1. Anesthesia was induced with 3% halothane; the animals were then tracheotomized, immobilized with tubocurarine chloride (0.5 mg · kg⁻¹) and artificially ventilated with 70% N₂O in oxygen. For continuous blood pressure (BP) recording and for blood sampling one femoral artery was cannulated. Intravenous (i.v.) injections of bicuculline and donor blood were given via a catheter in one femoral vein. The electroencephalogram was recorded from gold-plated bolts inserted in the skull bone. Rectal temperature was maintained close to 37 °C by external heating.

After the operative procedure was completed, the animals were given heparin i.v. They were maintained at steady state for 15–20 min after withdrawal of halothane. The arterial blood pressure was reduced from 150–165 mm Hg to 100–120 mm Hg by withdrawal of arterial blood. This was done to diminish the severity of early ictal hypertension. Bicuculline hydrochloride (Sigma Chemical Co.) dissolved in 0.1 N HCl and brought to pH 4–5 with 1 N NaOH was then injected i.v. in a dose of 1.2 mg · kg⁻¹ body weight.

After 5 min of seizure activity N₂O in the gas mixture was changed to N₂. Ventilation and O₂ supply were adjusted to keep an O₂ tension above 100 mm Hg. Arterial blood pressure, which tended

Table 1. Physiologic parameters in the different status epilepticus groups

	Arterial pressure mm Hg		Arterial pO ₂ mm Hg		Arterial pCO ₂ mm Hg		Arterial pH	
	A	B	A	B	A	B	A	B
Status epilepticus 2 h n = 13	127 ± 3	118 ± 3	136 ± 5	116 ± 6	27.4 ± 1.4	43.1 ± 1.5	7.000 ± 0.018	6.983 ± 0.033
Status epilepticus 1 h n = 4	128 ± 6	115 ± 2	131 ± 3	122 ± 3	27.0 ± 2.6	39.0 ± 2.0	7.089 ± 0.063	7.007 ± 0.011

The values are means + SEM

Time A: after 30 min of EEG seizures

Time B: after 90 min of EEG seizures for the 2-h group and at fixation for the 1-h group

to fall with prolonged periods of seizures, was maintained above 110 mm Hg by infusion of heparinized donor blood.

Sampling and Morphological Methods

After 1 or 2 h the rats with seizures were killed and their brains were fixed by vascular perfusion through a metal cannula placed in the ascending aorta after a brief rinse with saline. Twelve controls were similarly kept on artificial respiration for 2 h and then fixed in the same way.

The choice of fixatives was based on the following considerations. To make correct structural interpretations fixation must be done in a way that minimizes distortion of cells and organelles. Furthermore, dissolution of cellular components should be kept at a minimum. In this respect glutaraldehyde, alone or combined with formaldehyde, has been found the best for preservation of the CNS (Peters 1970; Palay and Chan-Palay 1974). Furthermore, during the primary fixation in glutaraldehyde the osmotic conditions are of great importance, in particular the vehicle osmolality (Kalimo 1976). Since cell volume changes do occur in our model, two different osmolalities of the buffer were used to exclude the possibility of fixative-induced artifacts:

(a) Glutaraldehyde 3% in 0.10 mol $\cdot$ l⁻¹ phosphate buffer pH 7.4, 37°C (seven rats 2 h; four rats 1 h);

(b) Glutaraldehyde 3% in 0.15 mol $\cdot$ l⁻¹ phosphate buffer, pH 7.4, 37°C (three rats 2 h).

To make comparisons with some previous studies (Meldrum and Brierley 1973; Blennow et al. 1978) the following fixative was used in some experiments:

(c) Formaldehyde, glacial acetic, and absolute methanol (David 1955) in ratios 1:1:18, pH 3.4, 37°C (three rats 2 h).

We allowed at least 250 ml of the glutaraldehyde fixatives to pass the vascular bed and about 1,500 ml of the FAM fixative for 20 min. Perfusion pressure was 135 mm Hg. After perfusion the brain was left *in situ* for at least 45 min; it was then removed and stored in the same fixative used until processed for microscopy. Efficiency of fixation was evaluated by absence of blood in vessels.

One hemisphere of the brain was coronally sectioned and embedded in plastic (JB-4, Polysciences Inc., Warrington, PA 18976, USA, or EFL-67, Serva Feinbiochemica, Heidelberg, FRG). From the other hemisphere samples were taken for EM and for embedding in paraffin. The JB-4 and EFL-67 embedded sections were stained with hematoxylin-eosin (HE) and with toluidine blue and the paraffin sections with HE and cresyl violet.

The cortical tissue blocks (Fig. 1) for EM were sectioned coronally into 230 μ m slices with a Sorvall TC-2 tissue sectioner. The slices were postfixed in 1% osmium tetroxide in cacodylate buffer, stained en bloc with 2% uranyl acetate in maleate buffer, dehydrated in a graded series of ethanol, and embedded in epon. Semithin sections stained with toluidine blue were used for the selection of areas for thin sections, which were double stained with uranyl acetate and lead citrate and examined in a JEM 100 C electron microscope.

To assess the extent of neuronal damage, the number of injured neurons was determined by counting every neuron with the aid of an eyepiece graticule at 40 $\times$ magnification in 250 μ m wide strips of cerebral cortex, extending perpendicularly from the cortical surface to the underlying white matter. Each strip contained 70–100 neurons which were classified into different categories.

Results

Cardiorespiratory Physiology

As described in earlier studies (Meldrum and Nilsson 1976; Chapman et al. 1977; Blennow et al. 1978)

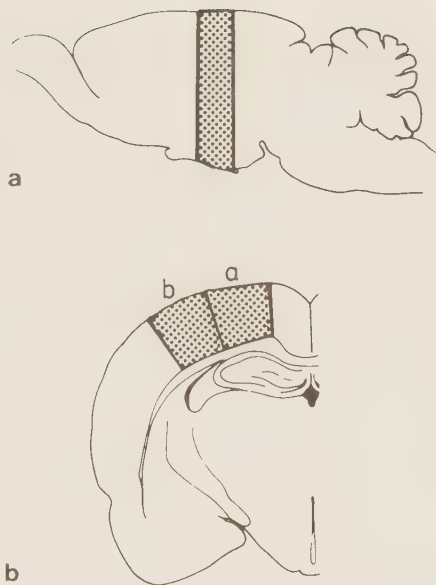


Fig. 1a, b. The sampling for electron microscopy. The brain was sectioned coronally (**a**) and multiple coronal sections were taken from areas **a** and **b** (**b**). This region corresponds to that sampled for biochemical analyses in our laboratory (e.g., Chapman et al. 1977; Folbergrová, Ingvar, and Siesjö, *in prep.*)

arterial blood pressure rose with the onset of EEG discharge, in this series to 180–215 mm Hg. After 5–15 min, blood pressure slowly declined and, after about 30 min, infusion of withdrawn blood was required for maintenance of blood pressure above 110 mm Hg. In animals in which status epilepticus was sustained for 2 h heparinized donor blood had to be infused to maintain the blood pressure until the end of the experiment. The amount given was 4–8 ml.

Mean values for the physiologic variables recorded after 30 min and at the end of the seizure period are summarized in Table 1. The values are in accordance with earlier results from this laboratory (Blennow et al. 1978).

EEG Changes

The onset of seizures was characterized by rapid irregular spikes followed by spikes of greater amplitude and rhythmicity. This pattern was then changed into one with bursts of rapid irregular spikes lasting 1–30 s separated by periods of EEG flattening of 1–10 s duration (*cf.*, Chapman et al. 1977).

Morphology

Controls. Our controls fixed with glutaraldehyde appeared in both LM and EM such as generally considered normal (Peters et al. 1976).

Status Epilepticus — Glutaraldehyde Fixation. Macroscopically, none of the brains showed any deviation from the controls. Animals with evident subarachnoid hemorrhage were excluded from the study. In a few animals a minimal amount of blood was present in the subarachnoid space, but structurally these brains differed in no way from those in which no visible bleeding had occurred. The brains were all firmly fixed and had a yellow tan color as is usually seen in well fixed glutaraldehyde-treated tissue.

All rats with status epilepticus for 1 or 2 h showed marked changes in the cerebral cortex with only minor interanimal variations. As expected, the lesions were somewhat less pronounced after 1 h as verified by the cell counts (Table 2). No difference could be detected between fixatives of different osmolarity. The main structural alterations in the cortex were nerve cell changes and status spongiosus (Fig. 2a–c).

Nerve Cell Changes. On the basis of the LM appearance the neurons were classified into four different categories as described in Table 2. The first category includes both normal neurons and neurons with minor changes in their shape or staining qualities. EM studies verified that these cells lacked prominent structural alterations. However, only about one half and one third of the neurons fell in this category after status epilepticus for 1 or 2 h, respectively.

The most frequent neuronal abnormality (injury type 1) in LM was characterized by a change in staining quality resulting in darker than normal neurons often with a shrunken cytoplasm, and surrounded by perineuronal vacuoles (Figs. 2b and 3a). They were most abundant in the areas showing status spongiosus, and their relative number increased from 40–55% as the duration of status epilepticus increased from 1–2 h.

The type 1 injured neurons were by LM further divided into two subpopulations, 1a and 1b, which differed from each other with regard to our possibility of identifying the nucleus. In type 1a neurons the

nucleus could be distinguished from the cytoplasm, but in type 1b the cells were so dark that internal structures could not be discriminated from each other (Fig. 3a). These two subpopulations occurred together in the same areas but type 1b neurons were less numerous than the other variety.

The condensed and darkly staining type 1a neurons were also identified by EM (Fig. 4). Such cells showed a slight dilatation of the endoplasmic reticulum and contained some vacuoles in their cytoplasm. There was little indication that these vacuoles represented ballooned mitochondria. Instead, the mitochondria looked either normal or showed only slight loss of inner matrix. These cells had an increased electron density of the nucleus; there was some clumping of the chromatin and infoldings of the nuclear membrane as in type 2.

Type 1b injured neurons (Fig. 5a and b) had a very electron dense cytoplasm with multiple groups of vacuoles. The origin of these vacuoles could not be determined with certainty. Some of these may be dilated mitochondria, yet most of them were so small that a mitochondrial origin is unlikely. Sometimes the vacuoles were grouped together in such a way that they most probably were dilated Golgi cisternae (Fig. 5a). The ribosomes as well as RER cisternae were often tightly packed (Fig. 5b). These changes extended into the dendrites. The barely visible nucleus was almost as dense as the cytoplasm and the chromatin appeared to be aggregated into large clumps. Often a massive accumulation of transmitter vesicles in axon terminals making synaptic contact with the pathologically altered type 1a and 1b neurons was noticed (Fig. 5b).

About 7–9% of the cortical neurons showed fairly normal staining characteristics but had slit-formed cytoplasmic vacuoles sometimes extending into the dendrites (Figs. 2c and 3b). These type 2 injured neurons were particularly abundant in the deeper cortical layers. Electron microscopy showed that most slit-formed vacuoles represented widened portions of rough endoplasmic reticulum (RER, Fig. 6a–c) including the part of RER that forms the nuclear envelope (Fig. 6b). Apart from the large vacuoles the type 2 neurons commonly contained numerous small vacuoles, some of which were apparently dilated Golgi vesicles or cisternae. Most often mitochondria looked normal, but rarely some type 2 neurons contained,

Fig. 2. a Cerebral cortex after 2 h of status epilepticus. The tissue is markedly edematous, with a spongy appearance of layer 3 and to a lesser extent in layers 5 and 6. Deep in layer 3 many condensed neurons (type 1 injury) and in layer 5 vacuolated neurons (type 2 injury) are visible. The outermost cortical layers remain quite compact. Epon + toluidine blue, $\times 130$. **b** Higher magnification of the status spongiosus in layer 3 after 2 h of status epilepticus. There are type 1a (arrows) and 1b (arrowheads) injured neurons surrounded by perineuronal vacuoles, and the neuropil shows generalized sponginess. Epon + toluidine blue, $\times 500$. **c** Higher magnification of the cortical layer 5 after 2 h of status epilepticus. The sponginess is less severe than in layer 3. The neurons display the type 2 injury with numerous intracytoplasmic vacuoles, which in one cell extend into the dendrite (arrow). Epon + toluidine blue, $\times 500$

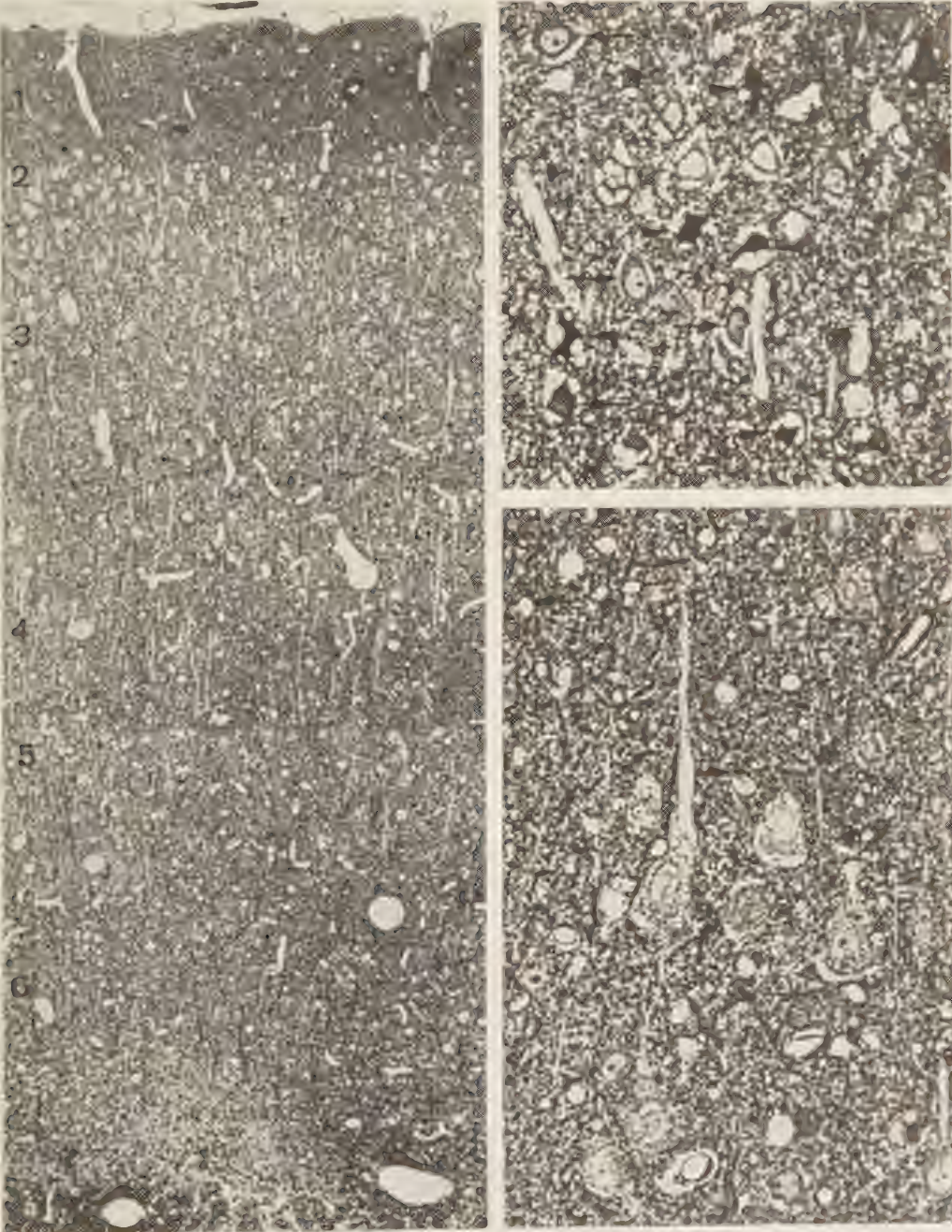


Fig. 2a-c

Table 2. Relative proportions of normal and different types of injured neurons in cerebral cortex of rats exposed to 1 or 2 h of bicuculline-induced status epilepticus

Group	Normal or minor change	Darkstaining, condensation of the nucleus and cytoplasm (type 1a)	Similar to type 1a but dark staining to such a degree that nucleus and cytoplasm cannot be distinguished (type 1b)	Normal staining but slit-formed cytoplasmic and perinuclear vacuoles (type 2)
	"	"	"	"
2 h status epilepticus. Fixation in 3% glutaraldehyde in 0.10 mol · l ⁻¹ phosphate buffer	36	44	12	7
2 h status epilepticus. Fixation in 3% glutaraldehyde in 0.15 mol · l ⁻¹ phosphate buffer	36	42	13	8
1 h status epilepticus. Fixation in 3% glutaraldehyde in 0.10 mol · l ⁻¹ phosphate buffer	49	33	8	9

amongst the normal mitochondria, a few vacuoles with crista-like material, which thus evidently represented swollen and partly disintegrated mitochondria (Fig. 6c). In the LM the nuclei of the type 2 injured neurons had slightly increased stainability (Fig. 3b) and, correspondingly, in EM the chromatin appeared slightly coarser than normally (Fig. 6a). In many type 2 injured neurons ribosomal polysomes disintegrated into single ribosomes (Fig. 6a and b).

Status Spongiosus. A most prominent alteration in all rats was status spongiosus particularly in the cerebral cortical layer 3 (Fig. 2a and b). As the changes progressed also the deeper layers 5 and 6 became spongy, but even then the outermost layer remained faintly stained. Electron microscopy showed that this pattern was formed by swollen glial processes, most probably of astrocytic origin. Very often they had a perivascular position and were also seen close to altered neurons especially of types 1a and 1b (Figs. 3a, 4, and 5a). Besides, swollen astrocytes were commonly seen in the otherwise fairly normal appearing neuropil. Many astrocytes had also a somewhat swollen nucleus with evenly dispersed chromatin (Fig. 7).

Status Epilepticus — FAM Fixation. In our hands FAM fixation preserved the structure of the tissues so poorly even in the control material that meaningful evaluation of the status epilepticus induced changes was considered impossible.

Discussion

As remarked in the Introduction, it has been difficult to exclude the possibility that the neuronal changes observed after sustained epileptic seizures in human are caused by cellular hypoxia. At first sight, it would appear that previous animal experiments prove that neuronal cell damage may occur in the absence of cellular hypoxia. Thus, although cerebral metabolic rate increases markedly the blood flow increases as well (Meldrum and Nilsson 1976). Furthermore, the phosphorylation state of the adenine nucleotide system is upheld close to control values (Chapman et al. 1977). Finally, curtailment of cerebral oxygen supply failed to exaggerate the lesions (Blennow et al. 1978).

In spite of this seemingly convincing evidence we cannot exclude that cellular energy failure contributes to the lesions observed. This is due to the facts that sustained seizures have been found to be accompanied by a mismatch between blood flow and metabolic rate in selectively vulnerable areas (Ingvar and Siesjö, in prep.) and that such areas (neocortex and hippocampus) show metabolic changes that are absent in "resistant" areas, such as the cerebellum (Folbergrová, Ingvar, and Siesjö, in prep.). Thus, the possibility exists that hypermetabolic cells develop a more extensive energy failure than that observed when tissue in bulk is analysed for metabolic variables. In other words, the lesions observed may be secondary to the combination of an excessive metabolic rate and moderate energy

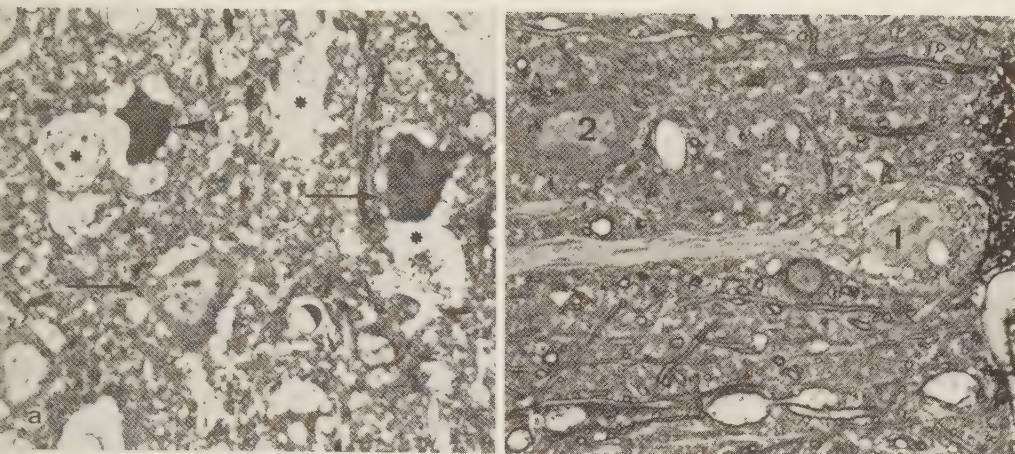


Fig. 3. **a** The type 1 injured neurons are usually triangular with condensed cyto- and karyoplasm and perineuronal vacuolation, which gives the cell a crenated outline. In type 1a neurons (arrows) the nucleus can still be discerned whereas in the more severely condensed type 1b neurons (arrowhead) this is no longer possible. Swollen watery astrocytes (*) are seen in connection with the perineuronal vacuoles and in the spongy neuropil. Epon + toluidine blue, $\times 1,050$. **b** A characteristic type 2 injured neuron (1) from layer 5 after 1 h of status epilepticus. There are many slit-formed, often perinuclear vacuoles in the cytoplasm, but no condensation of the cell. Virtually, no sponginess is observed in the surrounding neuropil and another neuron (2) appears intact. The picture exemplifies the less severe changes in deeper cortical layers after only 1 h of status epilepticus. Epon + toluidine blue, $\times 1,050$

failure. Obviously, if a restriction of oxygen supply does not exaggerate the lesions (Blennow et al. 1978) this could be due to a concomitant reduction in cellular metabolic rate (cf., Blennow et al. 1979).

The histopathologic changes observed in the present study were of two main types: (1) status spongiosus, principally in the middle cortical layers and (2) neuronal alterations characterized by intracellular vacuoles and/or condensation of cells with darkly staining cytoplasm. Status spongiosus, usually attributed to edema, has previously been observed in human autopsy material of epileptic patients (Scholtz 1959; Norman 1964). EM studies of brains from animals with epileptic seizures induced by methionine sulfoximine (Harris 1964), strychnine (Luse et al. 1964), or pentylene-tetrazole (de Robertis et al. 1969) suggest that this spongy state may be due to swelling of both astrocytic processes and of neuronal endoplasmic reticulum. In the article by Luse et al. (1964) the swollen cells were considered to be oligodendroglial. However, later studies suggest that the cells interpreted to be oligodendroglia were in reality astrocytes. Furthermore, following local injection of kainic acid — an excitatory, toxic analogue of the putative neurotransmitter glutamic acid — extensive swelling of neuronal RER was seen in those cerebellar cells, which presumably have receptors for glutamic acid (Herndon 1978).

The present results confirm that the light-microscopic vacuolation is of extra- and intraneuronal origin. Thus, condensed neurons of the type 1 injury were regularly surrounded by swollen cellular processes of apparent astrocytic origin. Besides, these swollen processes were frequently seen perivascularly, and the connection of these processes with the astrocyte soma often became apparent in the epon-sections (Fig. 3a).

Electron microscopy indicated that most of the intraneuronal vacuoles in the type 2 injured neurons represented dilated cisternae of the endoplasmic reticulum. Only solitary swollen mitochondria were encountered in some type 2 injured neurons, but no extensive ballooning of neuronal mitochondria had occurred. Furthermore, the mitochondria in the condensed neurons (type 1a) were usually not swollen. Our results therefore fail to support the conclusion of Meldrum and Brierley (1973) that status epilepticus is associated with the same type of microvacuolation, i.e., extensive swelling of mitochondria, as is observed in hypoxia-ischemia (see Introduction).

A specific type of nerve cell injury called "reactive change" (Ito et al. 1975; Bubis et al. 1976) or "selective chromatolysis" (Brown et al. 1979) has been observed in gerbils following ischemia induced by carotid artery occlusion. This type of injury was suggested by Brown et al. (1979) to be a consequence of "epileptic" activity characteristic of this species following ischemia. Our

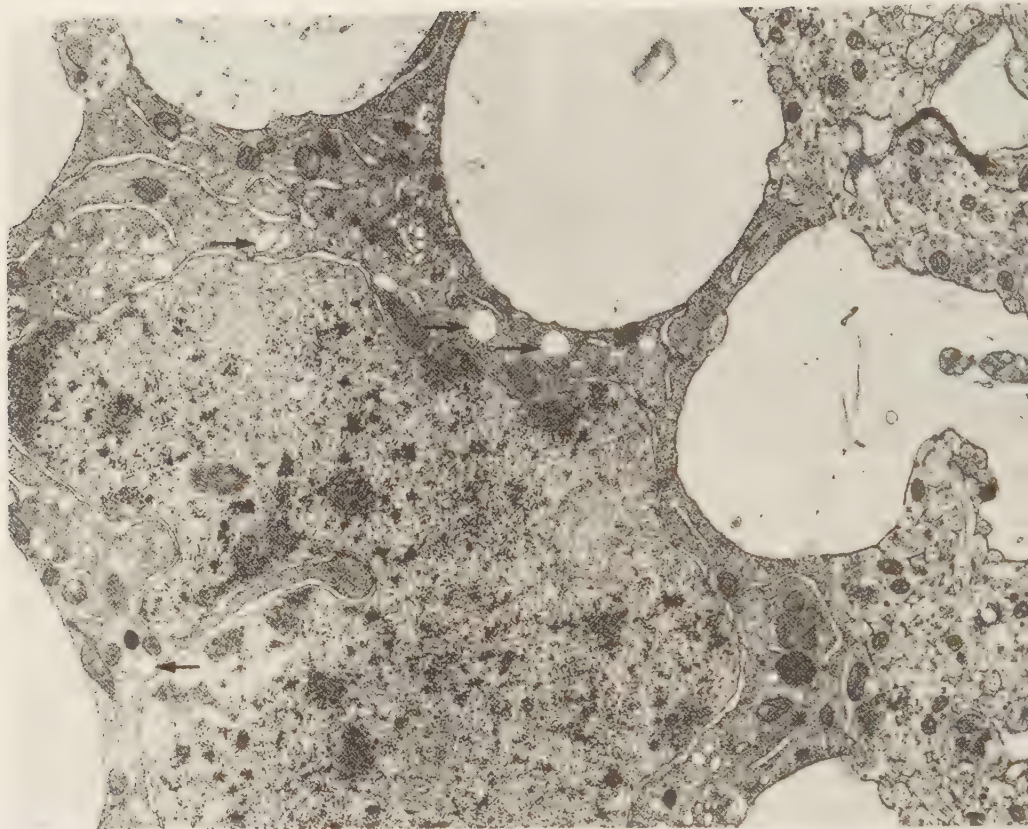


Fig. 4. A type 1a injured neuron from cortical layer 3 after 2 h of status epilepticus. The cyto- and karyoplasm are condensed and large vacuoles of apparent astrocytic origin bulge into the cytoplasm. The mitochondria appear intact, and the RER show few signs of swelling. A few single membrane bound vacuoles (arrows) are seen in cytoplasm. $\times 15,600$

type 2 injured neurons bear some resemblance to the "selective chromatolysis" change in respect to the cytoplasmic vacuolation. However, Brown et al. (1979) suggested that the larger vacuoles are derived from swollen mitochondria. As discussed above, this differs from our findings. As in the "selective chromatolysis" change we also observed disruption of polysomes into single ribosomes in many type 2 and 1a injured neurons, which agrees well with previous biochemical results following electrically induced seizures (see Wasterlain 1977). The crowding of organelles characteristic of "selective chromatolysis" was not seen in our material. It should be emphasized, though, that the experimental models are dissimilar, ours being overt status epilepticus, whereas "selective chromatolysis" is seen after ischemia-induced "epileptic" activity of variable intensity and duration.

In view of the similarity between the neurons of type 1a and b and the "dark cells" described by Cammermayer (1978) it is tempting to regard the changes as artifactual. The following facts argue against this possibility. First, neurons of such shape and staining characteristics were never observed in control animals. Second, patent capillaries indicate good perfusion of the fixative and the intact structure of most mitochondria in type 2 and 1a neurons is difficult to reconcile with poor fixation. Third, the number of type 1a and 1b neurons increased with increasing duration of seizures. Fourth, virtually all type 1a and most type 1b neurons disappeared during recovery after the seizure (unpubl. observ.).

Darkly stained, condensed neurons similar to the type 1a and b injured nerve cells are seen also in other types of injury, e.g., in severe hypoglycemia (type

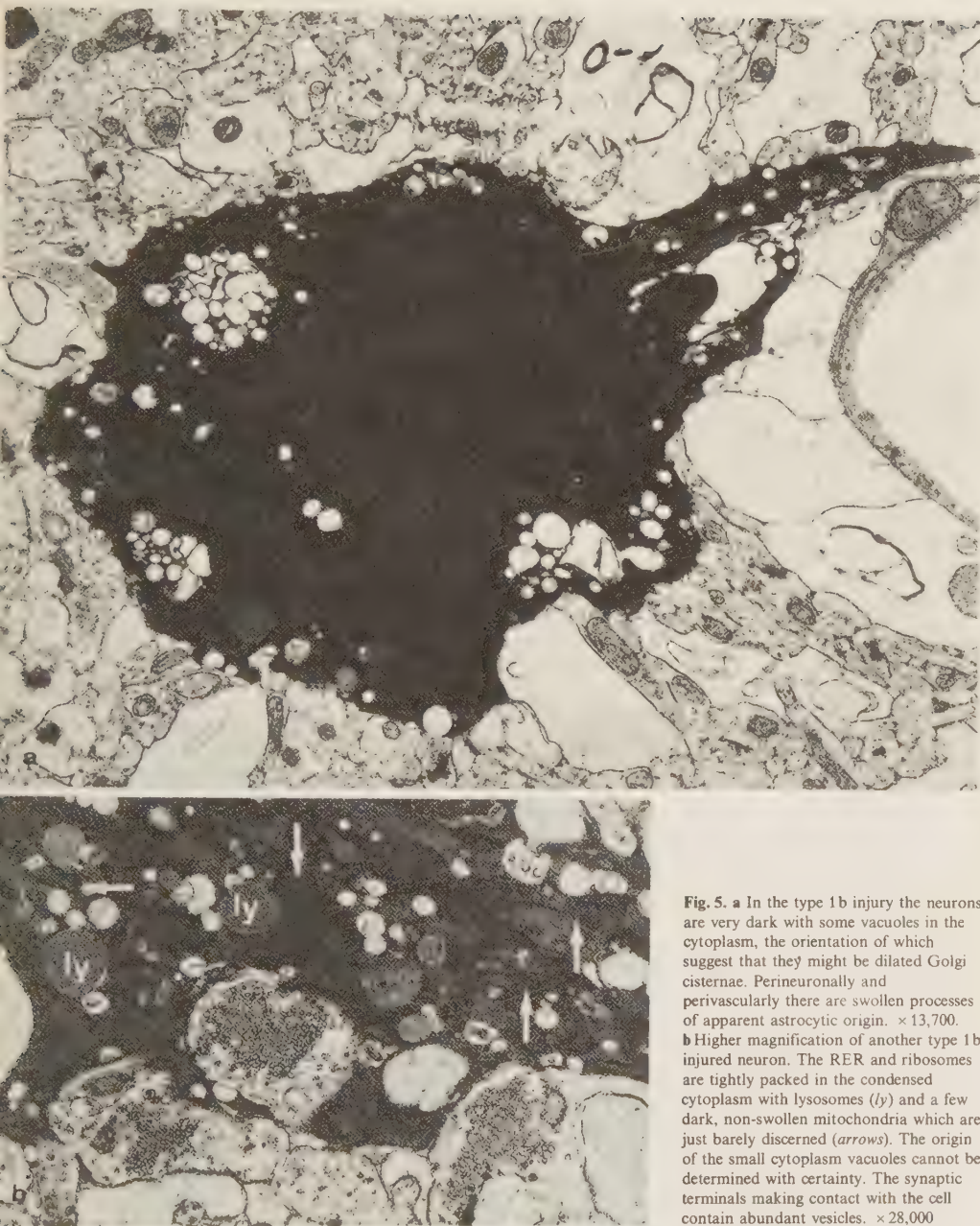


Fig. 5. a In the type 1b injury the neurons are very dark with some vacuoles in the cytoplasm, the orientation of which suggest that they might be dilated Golgi cisternae. Perineuronally and perivascularly there are swollen processes of apparent astrocytic origin. $\times 13,700$. **b** Higher magnification of another type 1b injured neuron. The RER and ribosomes are tightly packed in the condensed cytoplasm with lysosomes (*ly*) and a few dark, non-swollen mitochondria which are just barely discerned (*arrows*). The origin of the small cytoplasm vacuoles cannot be determined with certainty. The synaptic terminals making contact with the cell contain abundant vesicles. $\times 28,000$

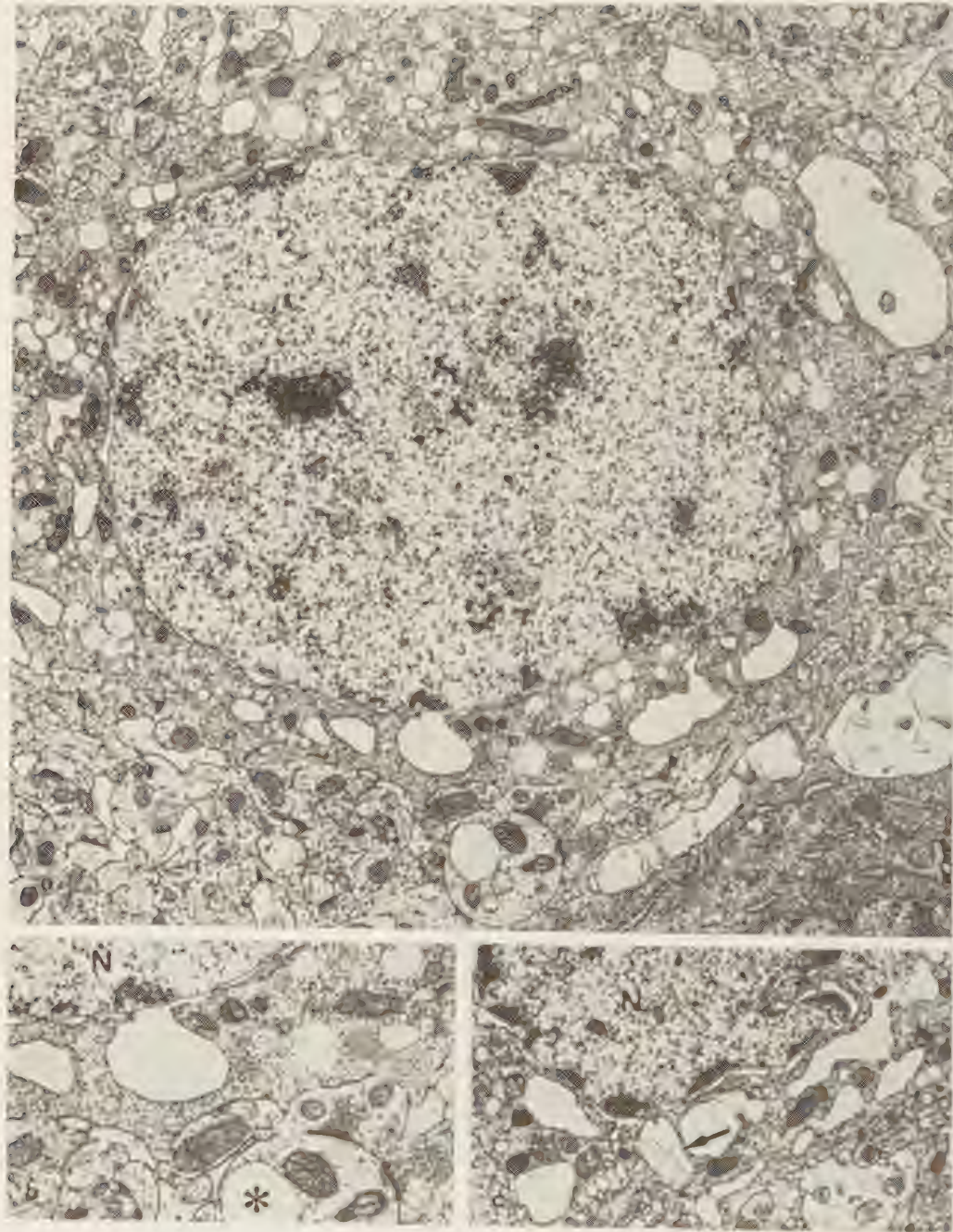


Fig. 6a-c

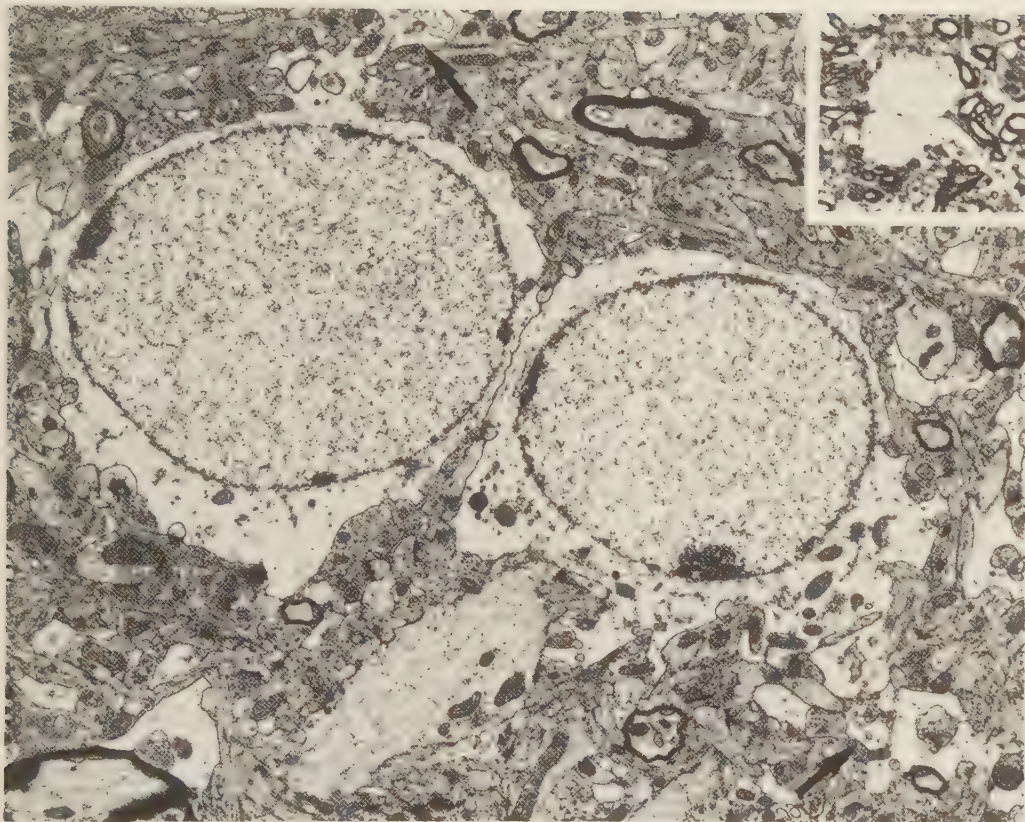


Fig. 7. Astrocytes from cortical layer 5 after 1 h of status epilepticus. their nuclei are somewhat distended but the chromatin is finely dispersed. Their cytoplasm appears watery and their processes (arrows) can easily be followed in the otherwise quite compact neuropil. *Inset:* epon + toluidine blue, $\times 1,200$; electron micrograph, $\times 8,000$

injured neurons. Agardh et al. 1980; Kalimo et al. 1980). This change was suggested to be an injury non-specific to hypoglycemia, a suggestion which is corroborated by the present results. Possibly, the injury is related to the tissue edema (see below).

At present, definite conclusions cannot be drawn regarding the reversibility of the nerve cell changes, but preliminary results of recovery experiments indicate that most of the changes observed are reversible (unpubl. observ.).

The spongy state in cortical layers 3 and 4 is most probably due to local edema and redistribution of the fluid with accumulation into the swollen astrocytes and RER cisternae of the type 2 neurons and loss of fluid from the type 1a and b neurons. The fact that the spongy state disappears as do type 2 and 1a neurons during recovery (preliminary data) is well explained on the basis of the disappearance of the edema fluid. This view is corroborated by the fact that the severity of the spongy state can be diminished by minimizing the

Fig. 6. **a** A type 2 injured neuron from cortical layer 5 after 1 h of status epilepticus. EM reveals that the cytoplasmic vacuoles are dilated ER cisternae. Mitochondria are not swollen, rather their inner matrix is slightly condensed. Nuclear chromatin is somewhat coarser than normally. The perineuronal cell processes are not swollen, except around the other neuron at the lower right which is slightly condensed, evidently an incipient type 1a change. $\times 10,700$. **b** A higher magnification of the same type 2 injured neuron showing the dilation of the part of RER forming the nuclear envelope as well as RER in a dendrite (*). N nucleus. $\times 16,000$. **c** Rarely, vacuoles (arrow) with crista-like remnants suggesting mitochondrial origin were encountered in some type 2 injured neurons. Note that most mitochondria appear normal and most vacuoles are of RER origin. N nucleus. $\times 11,000$

rise of arterial blood pressure (preliminary data). The localization of the spongy state principally in the cortical layer 3, later spreading into layer 5, could be explained by the fact that these layers contain pyramidal cells with a high calcium conductance and an innate capacity for spontaneous firing, thus requiring a continuous inhibitory (GABAergic) tone (Schwartzkroin and Wyer 1980; Roberts 1980).

A scrutiny of the literature reveals that the histopathologic picture bears no simple relationship to seizure duration. The widespread changes observed in the present study after 1 h of seizure activity, although seemingly in accordance with results reported in the baboon (Meldrum et al. 1973, 1978), contrast with the negative findings reported by others (Purpura et al. 1960; Schwartz et al. 1970; Brown et al. 1980). It remains to be shown whether these latter results were due to the preparation used or to the mode of induction of seizures. That the drug used to induce seizures is critical is evident from the study of Luse et al. (1964). They observed neuronal alterations after 1–4 h of seizures induced by strychnine, but not by picrotoxin, caffeine, sodiumbenzoate, pentylenetetrazole, or thiocarbonyhydrazide. Even more subtle experimental differences may be of importance since Hicks and Coy (1958), examining rats and mice of two different strains after methionine sulfoximic seizures, found that vulnerability differed both between the species and between strains of the same species. It therefore seems necessary to study systematically the conditions modifying the cellular response to seizures, using the same species under rigidly standardized conditions.

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INFLUENCE OF SYSTEMIC FACTORS ON EXPERIMENTAL EPILEPTIC BRAIN INJURY

Structural changes accompanying bicuculline-induced seizures
in rats following manipulations of tissue oxygenation or
 α -tocopherol levels.

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Yngve Olsson and Bo K. Siesjö

Summary

A previous study from the laboratory showed that status epilepticus, induced by bicuculline administration to ventilated rats, produced astrocytic swelling and nerve cell changes ("type 1 and 2 injury") particularly in layer 3 and 5 of the neocortex (Söderfeldt et al. 1981). The type 1 injured neurons were characterized by condensation of cyto- and karyoplasm and the less common type 2 cells were characterized by swelling of endoplasmic reticulum including the nuclear envelope.

In the present light (LM) and electron microscopic (EM) study we explored whether changes in cerebral oxygen availability altered the extent or character of the cellular alterations. To that end, animals with 2 h of status epilepticus were made either hyperoxic (administration of 100% O₂), hypoxic (arterial pO₂ 50 mm Hg) or hypotensive (arterial blood pressure of either 70-75 or 50 mm Hg). Furthermore, we explored whether "oxidative" damage occurred by manipulating tissue levels of α -tocopherol, a known free radical scavenger.

Non-epileptic control animals exposed to comparable degrees of hypoxia or hypotension showed no or minimal structural alterations. In the epileptic animals the results were as follows. Hyperoxia did not change the quality or extent of the structural alterations previously observed in normoxic epileptic animals. Neither administration nor deficiency of vitamin E did modify this pattern of alterations. In hypoxia the extent of cell damage was the same or somewhat larger than in normoxic, epileptic animals. In addition, neurons often showed cytoplasmic microvacuoles due to swelling of mitochondria. The hypoxic animals also showed swelling of astrocytic nuclei with clumped chromatin. Changes similar to those observed in hypoxic animals also appeared in moderate hypotension (mean arterial blood pressure 50 mm Hg), whereas mild hypotension (70-75 mm Hg) did not change the character of the tissue injury from that seen in hyperoxic or normoxic epileptic rats.

In conclusion, the present results demonstrate that the neuronal cell damage that can be observed when the brain is fixed by perfusion after status epilepticus of 2 h duration is not exaggerated by hyperoxia or vitamin E deficiency nor is it ameliorated by a moderate restriction in cerebral oxygen supply or by vitamin E administration. If anything, hypoxia (or moderate hypotension) appears to increase the extent of damage and it clearly alters its ultrastructural characteristics. However, although the results fail to support the notion that epileptic cell damage is "oxidative", definite conclusions must await information on the cell damage that remains upon arrest of the epileptic activity.

INTRODUCTION

Brain edema ("status spongiosus") and "ischemic" nerve cell damage are generally considered to be the major structural alterations that can be seen in patients dying of status epilepticus (Scholz 1959, Norman 1964, Corsellis and Meldrum 1976). From studies on human neuropathological material it is difficult to evaluate the relative importance of primary factors related to the increased neuronal activity per se and to secondary factors such as hypoxia and arterial hypotension. This difficulty is accentuated by the fact that the paroxysmal neuronal activity leads to increased oxygen demands. It is conceivable, therefore, that even moderate hypoxia or hypotension could hazard cerebral oxygen supply.

In experimental studies with pharmacologically induced status epilepticus, physiological variables can be monitored and controlled. However, the effect of systemic factors on the neuropathological outcome remains controversial. On the one hand, it has been demonstrated that artificial ventilation and seemingly adequate oxygenation of arterial blood reduce the extent of brain damage (Meldrum and Brierley 1973). On the other hand, the results of another study suggested that neuropathological alterations were less severe when the cerebral oxygen availability was reduced by moderate arterial hypoxia or hypotension (Blennow et al. 1978). The results of the latter study would suggest that epileptic damage is due to the epileptic discharge per se, and that the extent of the damage depends on a plentiful oxygen supply, possibly because free radical mechanisms are involved.

A recent study from our group in which histo-pathological alterations during bicuculline-induced status epilepticus in the rat were evaluated both with high resolution light microscopy (LM) and with electron microscopy (EM), gave results that were seemingly discordant with previous ones (Söderfeldt et al. 1981). Thus, the LM results showed more extensive alterations than those published by Blennow et al. (1978). Furthermore, the EM studies failed to conform with previous notions that epileptic seizures give rise to the typical "ischemic" cell changes (Meldrum and Brierley 1973). Notably, little evidence was obtained that high-amplitude mitochondrial swelling occurs.

In this study we wished to investigate in greater detail the influence of cerebral oxygen availability on the extent and the type of structural damage that occur in the rat cerebral cortex during bicuculline-induced status epilepticus. To that end, we compared animals exposed to either hyperoxia or moderate hypoxia, as well as those rendered mildly or moderately hypotensive, before the animals were perfusion-fixed at the end of a 2 h seizure period. Furthermore, in order to gain additional information on the possibility that free radical mechanisms lead to "oxidative" cell damage, LM and EM studies were carried out on animals that were either fed a vitamin E-deficient diet, or were given sufficient vitamin E to appreciably raise the brain tissue levels of α -tocopherol.

MATERIAL AND METHODS

Animals and model

The experiments were carried out on male Wistar rats with free access to water and food until anesthesia was induced with 3% halothane. The animals were then tracheotomized, immobilized with tubocurarine chloride (0.5 mg.kg^{-1}) and artificially ventilated with 70% nitrous oxide (N_2O) and 30% oxygen (O_2). Femoral venous and arterial cannula were inserted and mean arterial blood pressure (MABP) was recorded polygraphically. The EEG was recorded from gold-plated bolts in the skull bone. Rectal temperature was maintained close to 37°C by external heating.

After the operative procedure was completed the animals were given heparin i.v. They were maintained at steady state for 15-20 min after withdrawal of halothane. The arterial blood pressure was reduced from 150-165 mm Hg to 100-120 mm Hg by withdrawal of arterial blood. This was done to diminish the severity of early ictal hypertension. Bicuculline chloride (Sigma Chemical Co), dissolved in 0.1 N HCl and brought to pH 4-5 with 1 N NaOH, was then injected i.v. in a dose of 1.2 mg.kg^{-1} body weight.

After five min of seizure activity, N_2O in the gas mixture was changed to N_2 (except in the hyperoxic group, see below).

The following experimental groups were investigated.

Controls with hypoxia alone

After maintenance at steady state for 20 min hypoxia was induced by reducing the oxygen concentration of the gas mixture (n=3). Arterial pO₂ was kept close to 50 mm Hg and arterial blood pressure was maintained above 100 mm Hg. These animals were ventilated on a gas mixture of N₂O and O₂-N₂ during the whole experiment.

Controls with severe hypotension alone

Twenty min after halothane withdrawal MABP was reduced to 50 mm Hg by bleeding and then maintained at this level. Arterial oxygen tension was kept above 100 mm Hg. These animals (n=4) were ventilated on a gas mixture of 70 per cent N₂O and 30 per cent O₂.

Controls fed on a vitamin E-deficient diet

As described below, five animals were fed on a vitamin E-deficient diet. In two of these, which served as controls, no seizures were induced. The animals were maintained normotensive and normoxic on N₂O:O₂ (70:30) until the perfusion-fixation.

Status epilepticus combined with hyperoxia

After five min of seizure activity the gas mixture was changed and the animals were ventilated on 100% oxygen. Also in this group (n=6) arterial blood pressure was kept above 100 mm Hg. In this and the forthcoming groups the brain was fixed by perfusion after 2 h of status epilepticus.

Status epilepticus combined with hypoxia

In four rats oxygen concentration in the gas mixture was reduced to give a pO₂ close to 50 mm Hg approximately 30 min after bicuculline injection. Arterial blood pressure was maintained above 100 mm Hg.

Status epilepticus combined with arterial hypotension

In four rats MABP was lowered by bleeding 5-20 min after onset of seizures and maintained at 70-75 mm Hg. Arterial oxygen tension was kept above 100 mm Hg. In four other rats the same procedure as above was carried out, but the blood pressure was lowered down to 50 mm Hg.

Status epilepticus following manipulation of tissue vitamin E-levels

Increased vitamin E levels were induced in standard animals fed on the commercial pellet food (n=4). A total amount of 1.5 g.kg^{-1} of vitamin E in aqueous solution (Ido-E $\text{\textcircled{R}}$, aquosum vet. Ferrosan, Malmö) was given i.p., divided into three single doses. The injections were given 15 h, 1 h, and just prior to the experiment, respectively.

In order to induce vitamin E deficiency, five animals were fed on a synthetic diet deficient in vitamin E, after the animals had been weaned. Details of the composition of the diet, as well as methods for measuring tissue levels of α -tocopherol, will be reported separately (Westerberg et al. in preparation). Vitamin E-deficient animals (n=3) were fixed by perfusion after 2h of status epilepticus.

Methods and sampling

After 2 h of status epilepticus, or after 2 h on respirator for the controls, a quick thoracotomy was performed and a metal cannula was placed in the ascending aorta. The perfusion was started with a brief rinse with saline followed by the fixative (3% glutaraldehyde in 0.1 mol.l^{-1} phosphate buffer, pH 7.4, 37°C , at a pressure of 135 mm Hg). We allowed at least 250 ml of the fixative to pass the vascular bed. After fixation the brain was left in situ for at least 45 min. It was then removed and stored in the fixative until processed for microscopy. Efficiency of perfusion was evaluated by the amount of remaining blood in the cortical vessels.

One hemisphere was coronally sectioned and embedded in plastic (EFL-67, Serva Feinbiochemica). From the other hemisphere, samples from two fronto-parietal cortical areas were embedded in epon for high resolution LM and EM (Fig. 1). The sections were

stained with toluidine blue. Each block for EM was sectioned into several 230 μ m slices with a Sorvall TC-2 tissue sectioner. The slices were postfixed in 1% buffered osmium tetroxide, en bloc stained with uranyl acetate, dehydrated in a graded series of ethanol and embedded in epon. Semithin sections stained with toluidine blue were used for high resolution LM and selection of areas for thin sections. These were double stained with uranyl acetate and lead citrate and examined in a JEM 100 C or a Zeiss EM 10 electron microscope.

In order to assess the extent of neuronal damage the number of structurally altered cells was determined by counting every neuron in 250 μ m wide strips extending perpendicularly from the cortical surface to the underlying white matter (Fig. 1). Each strip contained 70-100 neurons which were classified in different categories according to the same criteria as used in a previous study (Söderfeldt et al. 1981).

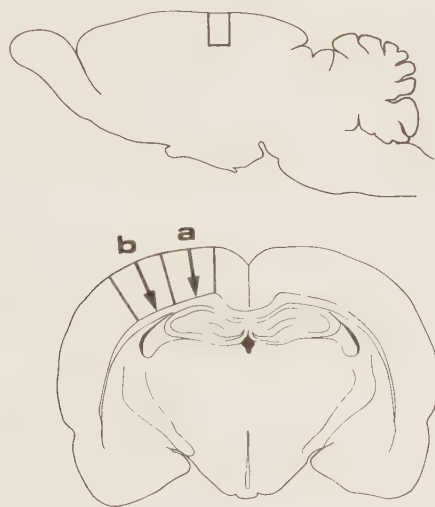


Fig.1 Sampling for microscopy. Multiple coronal sections were taken from areas a and b. Cell counts were performed in the strips indicated by the arrows.

RESULTS

Cardiorespiratory physiology

In all the groups with status epilepticus the initial changes were the same as described in earlier studies on the same model (Meldrum and Nilsson 1976; Chapman et al. 1977; Blennow et al. 1978; Söderfeldt et al. 1981). An initial rise of arterial blood pressure to 180–200 mm Hg was consistently observed. The pressure began to decline 5–15 min after the onset of seizures and the subsequent level was governed by experimental manipulations. In animals that were not deliberately made hypotensive, MABP was maintained above 100 mm Hg by infusion of donor blood.

Mean values for key physiological variables determined (a) after the selective manipulations had taken effect and (b) at the end of the experiment are summarized in Table 1. The values are consistent with those earlier published by Blennow et al. (1978).

Cerebral cortical levels of α -tocopherol

Biochemical changes observed in animals whose tissue α -tocopherol levels were manipulated will be reported in detail separately (Westerberg et al. in preparation). In summary, administration of vitamin E to standard animals raised brain tissue α -tocopherol levels from a control value of 18.6 ± 0.9 to 30.3 ± 2.7 $\mu\text{g/g}$ tissue. Animals fed on a synthetic diet with supplemented vitamin E had control levels of 12.2 ± 0.6 $\mu\text{g/g}$ tissue. In the vitamin E-deficient animals, the tissue levels were significantly reduced (7.3 ± 0.7 $\mu\text{g/g}$ tissue).

EEG changes

The onset of seizures was indicated by rapid, irregular EEG spikes with increasing amplitude and rhythmicity. This pattern was then changed into one with bursts of rapid irregular spikes lasting 1–30 s, separated by periods of EEG flattening of 1–8 s duration (cf. Chapman et al. 1977). In the group with moderate arterial hypotension there were periods of single spike or of polyspike and wave activity interspersed with bursts of fast spikes. The same pattern was also seen in the hypoxia group. Also in this respect the changes were the same as described by Blennow et al. (1978). Hypoxia and hypotension control animals had continuous EEG activity without epileptic discharge, as had the vitamin E deficient controls.

Table 1. Physiological variables in the different experimental groups.

	Arterial pressure mm Hg		Arterial pO ₂ mm Hg		Arterial pCO ₂ mm Hg		Arterial pH	
	A	B	A	B	A	B	A	B
Hypoxia n=3	140 +18 —	148 +12 —	49 +3 —	48 +2 —	34.5 +0.8 —	31.3 +0.9 —	7.463 +0.017 —	7.490 +0.012 —
Hypotension n=4	50 +0 —	50 + —	112 +5 —	120 +8 —	27.4 +2.0 —	28.9 +1.0 —	7.260 +0.070 —	6.981 +0.087 —
Status epilepticus combined with hyperoxia n=6	127 +4 —	116 +5 —	277 +28 —	298 +16 —	29.0 +1.7 —	35.6 +3.2 —	6.980 +0.041 —	6.985 +0.072 —
Status epilepticus combined with hypoxia n=4	118 +5 —	113 +5 —	49 +2 —	55 +3 —	24.0 +1.6 —	33.5 +2.2 —	7.030 +0.007 —	6.880 +0.030 —
Status epilepticus combined with mild hypotension n=4	73 +1 —	71 +1 —	148 +12 —	141 +15 —	21.0 +2.7 —	31.0 +3.3 —	6.988 +0.105 —	6.924 +0.014 —
Status epilepticus combined with moderate hypotension n=6	50 +0 —	50 +0 —	121 +5 —	114 +4 —	24.8 +3.2 —	33.5 +1.8 —	7.010 +0.005 —	6.793 +0.041 —
Status epilepticus combined with vitamin E deficiency n=3	127 +3 —	122 +3 —	137 +7 —	119 +3 —	29.6 +2.6 —	35.8 +2.0 —	7.231 +0.069 —	6.890 +0.069 —
Status epilepticus combined with vitamin E administration n=4	136 +3.0 —	107 +12 —	112 +2 —	102 +4 —	30.5 +3.1 —	35.1 +2.4 —	7.130 +0.032 —	7.138 +0.021 —

The values are means $\pm$ S.E.M. Time A: after 30 min of EEG seizures or after introduction of hypoxia/hypotension
 " B: " " " " " " " " " " " "

Morphology

The brains of animals subjected to hypoxia, hypotension or vitamin E deficiency without status epilepticus looked macroscopically normal. In LM no difference from what is generally considered normal could be detected (Fig. 2 a,b). In EM, two of the hypoxia and all of the hypotension controls appeared completely normal. In one of the hypoxic animals most structures were normal, but a few vessels were surrounded by slightly swollen astrocytic processes. These results thus demonstrate that this degree of oxygenation and blood flow reduction give no or minimal morphological changes in the cerebral cortex (cf. Bakay and Lee 1968).

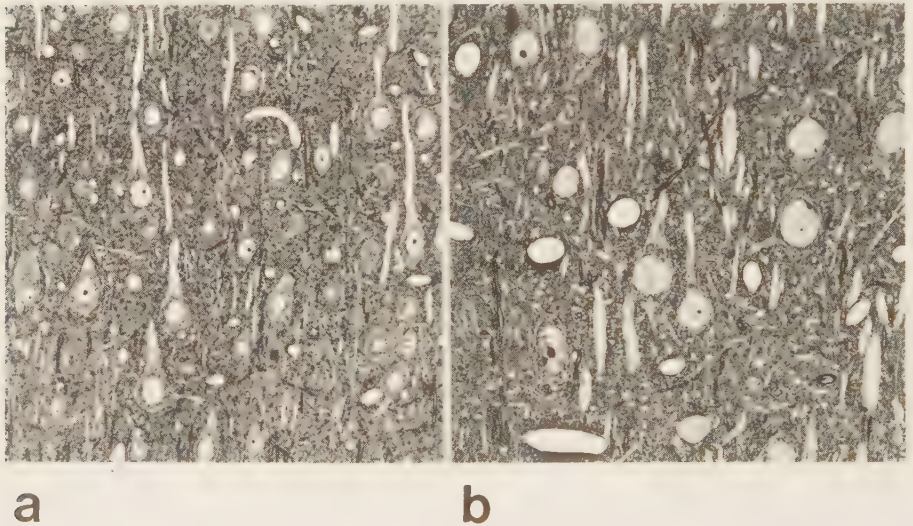


Fig.2 Sections from layer 5 in a control rat with hypoxia (a), and from layer 3 in a rat with moderate hypotension (b) alone. The structure shows no pathological changes. Epon. Toluidine blue. a. 290 x, b. 730 x.

The cortical areas from the epileptic rats were all judged to be adequately fixed. Thus, blood was absent in almost all cortical vessels. So few capillaries contained erythrocytes that they most probably did not interfere with the fixation of the tissue.

For comparison the characteristic changes of bicuculline-induced status epilepticus in normoxic and normotensive animals described in the previous study (Söderfeldt et al. 1981) are shortly presented here. In the cerebral cortex of normoxic and normotensive rats status epilepticus produced nerve cell changes, called type 1 and 2 injury (cf. Fig. 3-5), and astrocytic swelling. The type 1 injured neurons displayed progressive condensation of cyto- and karyoplasm. The less common type 2 injury was characterized by swelling of the endoplasmic reticulum including the nuclear envelope.

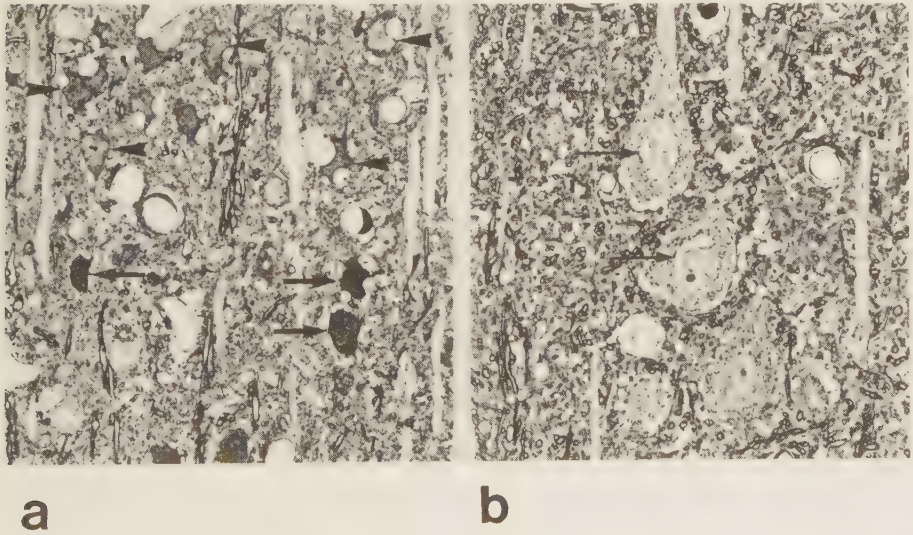


Fig.3. Status epilepticus combined with hyperoxia. The changes are the same as in normoxic and normotensive epileptic rats.

a. Several type 1a (arrows) and 1b (arrowheads) neurons from cortical layer 3.

b. Typical type 2 change in a large pyramidal neuron from layer 5. Note the large perinuclear vacuoles (arrows).

Epon. Toluidine blue. a. 730 x, b. 730 x.

Table 2. Proportions (%) of normal and injured neurons in fronto-parietal cerebral cortex of rats with status epilepticus combined with hyperoxia, hypoxia or hypotension (cf. Table 1). All the cells in a defined area were classified into 4 groups based on their LM appearance in epon-embedded toluidine blue stained sections. All values are means $\pm$ S.E.M.

Group	Normal or minor change	Darkstaining, condensation of nucleus and cytoplasm	Similar to 1a but darkstaining to such a degree that nucleus and cytoplasm cannot be distinguished	Normal staining but slitformed cytoplasmic and perinuclear
			Type 1a	Type 2
Status epilepticus combined with hyperoxia	45 $\pm$ 5.7	41 $\pm$ 2.1	5 $\pm$ 1.1	8 $\pm$ 1.1
Status epilepticus combined with hypoxia	38 $\pm$ 0.3	45 $\pm$ 2.2	10 $\pm$ 0.9	6 $\pm$ 1.0
Status epilepticus combined with mild hypotension	48 $\pm$ 3.2	41 $\pm$ 3.4	5 $\pm$ 1.8	5 $\pm$ 1.6
Status epilepticus combined with moderate hypotension	35 $\pm$ 3.0	43 $\pm$ 1.9	17 $\pm$ 2.6	4 $\pm$ 1.0
Status epilepticus combined with vitamin E deficiency	43 $\pm$ 3.3	43 $\pm$ 2.5	11 $\pm$ 2.6	3 $\pm$ 1.0
Status epilepticus combined with vitamin E administration	41 $\pm$ 2.2	44 $\pm$ 3.5	8 $\pm$ 1.0	7 $\pm$ 0.8

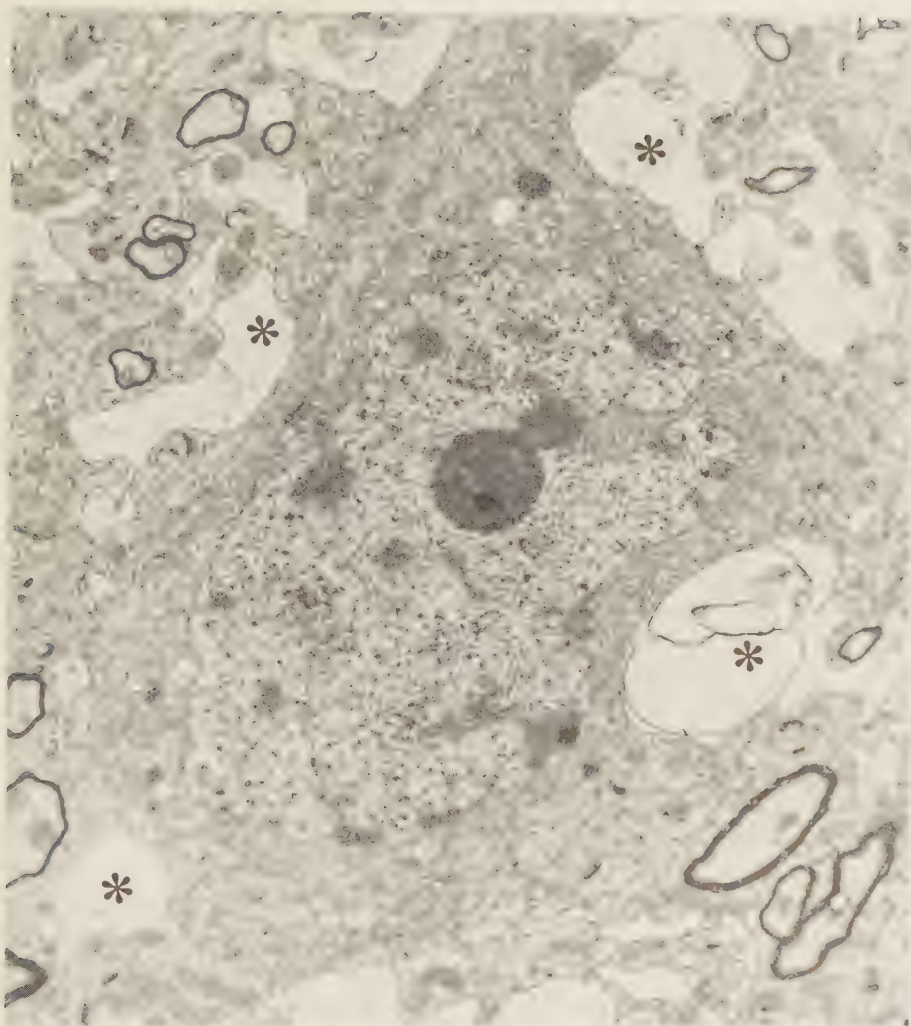


Fig.4 Status epilepticus combined with hyperoxia. This electron micrograph shows a typical type Ia neuron from cortical layer 3. Both the cyto- and karyoplasm are condensed and the cell is surrounded by swollen astrocytic processes (asterisks). Note that the mitochondria are not swollen. 15000 x.

Status epilepticus combined with hyperoxia

Animals in this group showed by LM nerve cell changes in the cerebral cortex of the same quality and distribution as in normoxic and normotensive rats with status epilepticus (Fig. 3 a,b), i.e. the predominant neuronal change was of type 1 (approximately 46% of all neurons) whereas the type 2 injured neurons comprised about 8% of all nerve cells (see Table 2).

Type 1 injured neurons were most frequent in cortical layer 3. A few of them were also present in layer 2 as well as in deeper parts of the cortex. By LM most of these cells did not have cytoplasmic vacuoles. In EM type 1 neurons showed condensation of cyto- and karyoplasm. the degree of this condensation varied among the type 1 neurons from slight (type 1a, Fig. 4) to severe (type 1b) in which the nucleus could not longer be discerned. The cytoplasmic organelles showed fairly few abnormalities. In most type 1a neurons mitochondria retained their compact structure and ER cisternae remained narrow. Type 1b had very dark cytoplasm, where organelles were hardly discernible, except for some clear vacuoles, most probably dilated Golgi cisternae.

Type 2 injury affected most often large neurons in cortical layers 5 and 6. Exceptionally they were present in more superficial layers as well. This injury was characterized in LM by multiple, large and elongated vacuoles in the perikaryon, often extending into the dendrites. In this cell type the nucleus was always visible but often deformed by perinuclear vacuoles (Fig. 3b). EM (cf. Fig. 5) proved that the vacuoles in fact were dilated ER cisternae including the part forming the nuclear envelope. Smaller vacuoles presumably originated from the Golgi apparatus. In general, the mitochondria were only slightly affected.

Besides nerve cell changes we observed a prominent astrocytic edema particularly in cortical layer 3. Within the layer the edema was often irregularly distributed. Layers 1 and 2 were consistently spared with the exception for a narrow subpial zone. The swollen astrocytic processes had a characteristic localization around vessels. Also type 1 neurons in the edematous areas were usually surrounded by such processes. The nuclei of the swollen astrocytes seemed to be slightly enlarged and their chromatin was in the deeper layers most often finely dispersed giving them a ground-glass appearance.

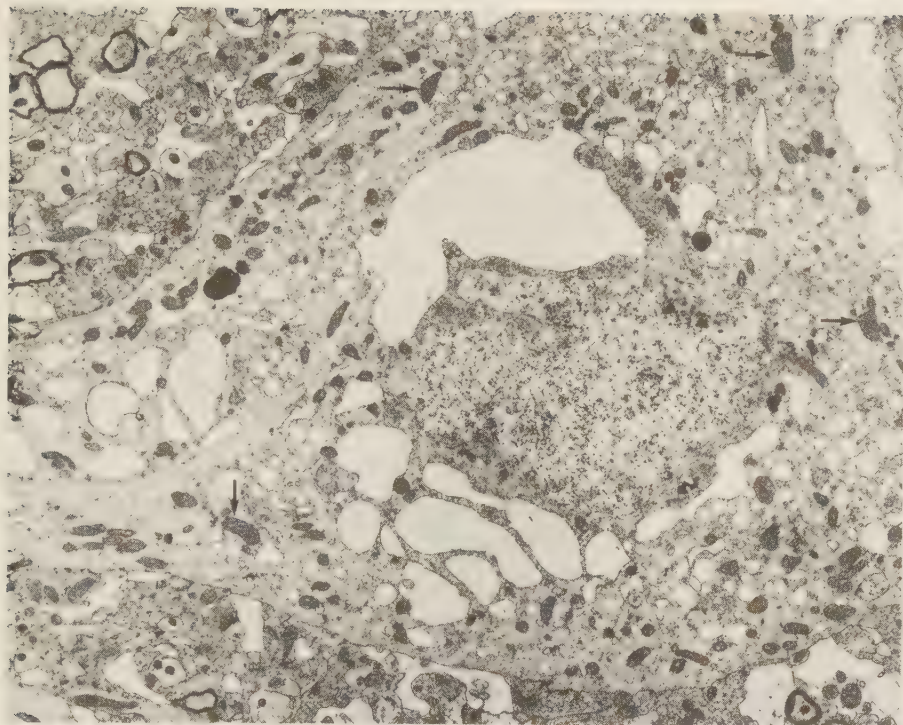


Fig.5 Status epilepticus combined with mild hypotension. This typical type 2 injured neuron is derived from layer 5. The cytoplasm contains numerous vacuoles formed out of dilated cisternae of the rough endoplasmic reticulum which extend also into the dendrite on the left. Some smaller vacuoles obviously originate from golgi cisternae. Mitochondria (some marked with arrows) are unaffected. 6400 x.

Status epilepticus and vitamin E

Vitamin E administration did not alter the extent or the character of the cellular changes as compared with those observed in normoxic (Söderfeldt et al. 1981) or hyperoxic (present material) animals. Likewise, vitamin E-deficient animals showed no exaggeration of the cell damage.

Status epilepticus combined with hypoxia

In general the distribution of the structural changes was similar to that in the previous group but the degree of injury appeared somewhat more severe as suggested also by the cell counts (Table 2). One of the two LM differences between this and the preceeding groups was the presence of microvacuoles in many type 1b neurons (Fig. 6). EM revealed that in such cells many mitochondria were enlarged and that the cristae were ruptured (cf. Fig. 7) though some type 1 injured neurons still had normal mitochondria.

The other significant structural difference was present in the nuclei of edematous astrocytes. The chromatin was sparse and clumped and the nuclei were definitely enlarged (Fig. 6,8).

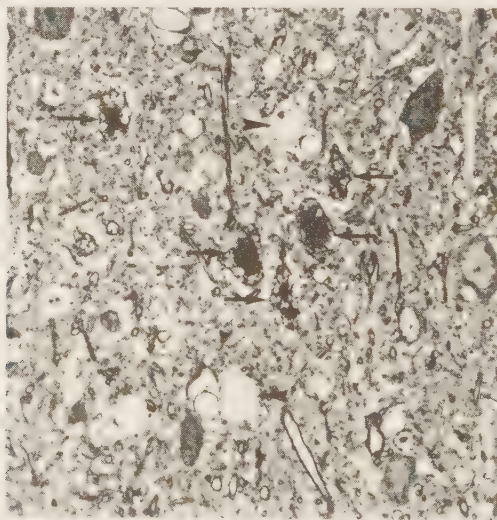


Fig.6 Status epilepticus combined with hypoxia. Section obtained from cortical layer 3. All the type 1b neurons are microvacuolated (arrows). Note the swollen appearance of the astrocytic nuclei with sparse and clumped chromatin (arrowheads).
Epon. Toluidine blue 730 x.

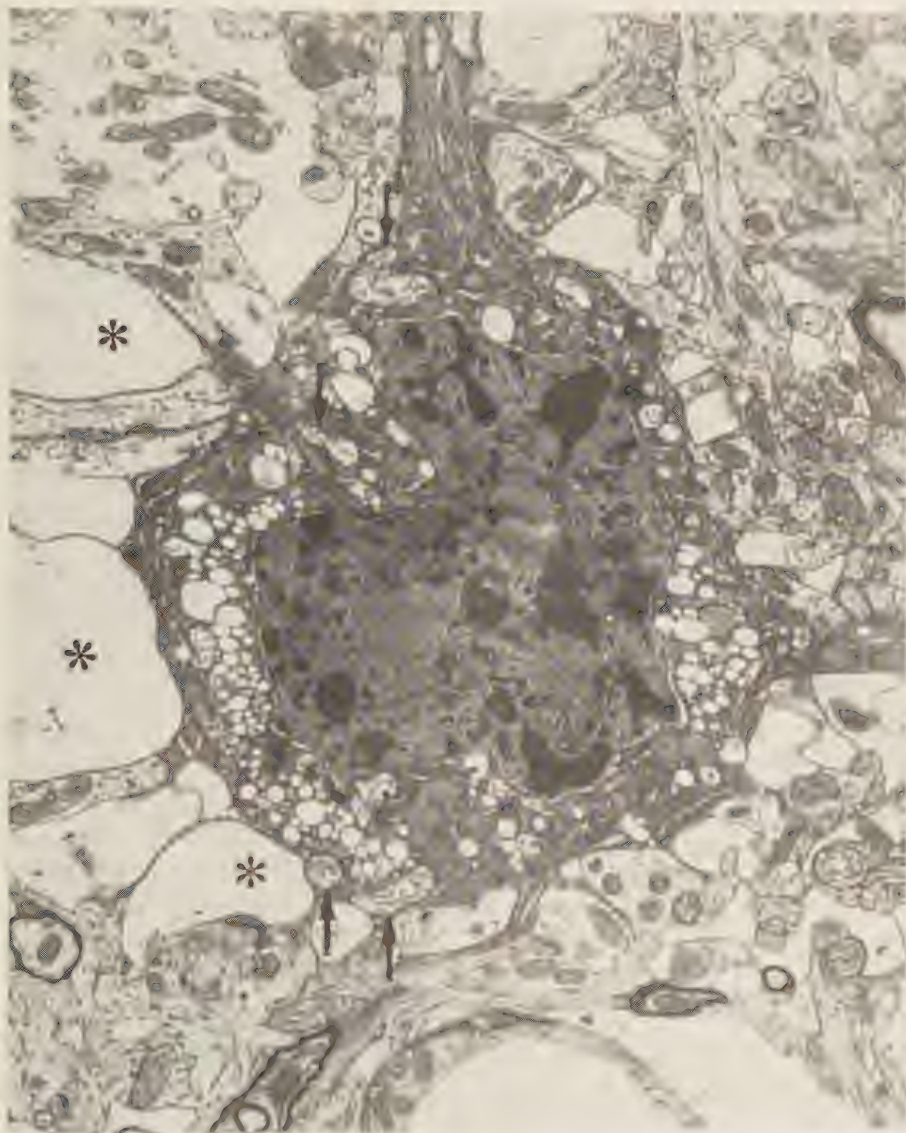


Fig.7. Status epilepticus combined with moderate hypotension. In this type 1b injured neuron (cortical layer 3) mitochondria (arrows) are swollen with disoriented cristae. Besides there are numerous smaller vacuoles, many of which are obviously dilated golgi cisternae. 9000 x.

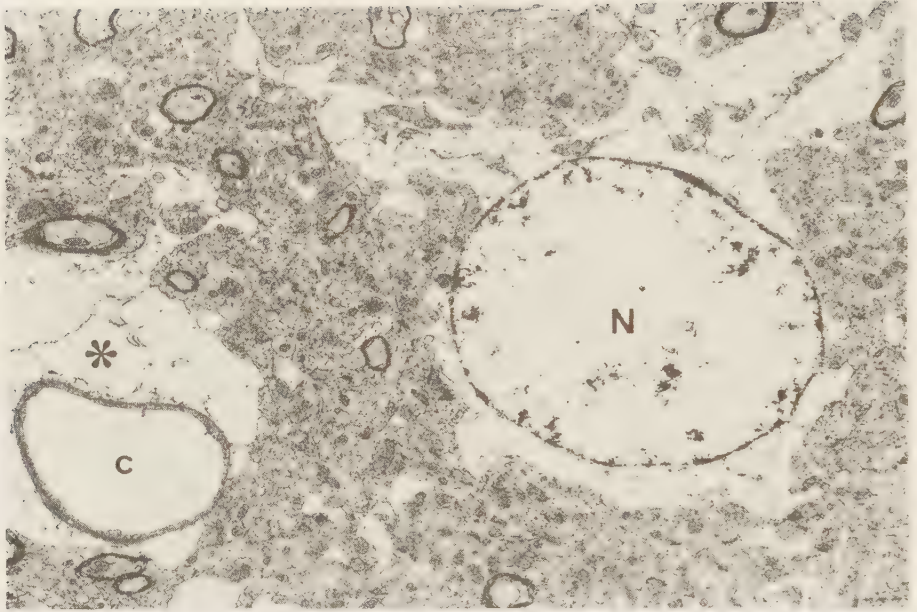


Fig.8 Status epilepticus combined with hypoxia. The astrocytic nucleus (N) is swollen with sparse and clumped chromatin. The cytoplasm is watery including the perivascular processes (asterisk) c = capillary. 5100 x.

Status epilepticus combined with hypotension

In epileptic rats exposed to moderate hypotension (MABP 50 mm Hg) the character and distribution of the injury in LM were nearly identical to those in the epileptic rats with hypoxia (Fig. 6). In one animal a focal lesion with edema and type 1 nerve cell changes was present in the deepest part of the cortex. By EM the changes were virtually the same as in the preceeding group. On the other hand, if the hypotension was only mild (MABP 70-75 mm Hg), the changes approached those in the hyperoxic as well as normoxic-normotensive rats with status epilepticus (Fig. 5).

DISCUSSION

In the present experiments the brains were fixed by perfusion after a 2 h period of continuous bicuculline-induced seizure, i.e. at a time when paroxysmal activity was still present. The experimental conditions were, therefore, somewhat different from those of Brown et al. (1980), who studied the neuropathological outcome of prolonged bicuculline-induced seizures in cats by perfusion-fixation of tissue 1-6 h following pharmacological arrest of the seizure activity. However, our experimental conditions are similar to those Meldrum and Brierley (1973) in the baboon and directly comparable to those of Blennow et al. (1978). There are differences in methodology, though. In the present study, glutaraldehyde was chosen as the fixative since the formaldehyde, acetic acid methanol mixture FAM (David 1955), used by Meldrum and Brierley (1973) and by Blennow et al. (1978) does not give adequate fixation for EM. Also at the LM level glutaraldehyde together with epon embedding gives a better preservation of cellular details and a higher resolution than the technique based on FAM fixation.

The question arises to what extent changes observed in our material are artefactual due to improper tissue fixation. It must also be asked whether the cellular changes observed have their counterparts in intravital changes. There are reasons to believe that the structural alterations observed presently are not due to improper tissue fixation. Thus, macroscopically the brains were firm in consistency and brownish-yellow in colour. Furthermore, on microscopic examination the vessels were patent and free of blood constituents.

It is more difficult to assess whether the changes observed in the present study have their intravital counterparts, since such changes may depend on the reaction of a damaged tissue to the fixation and the further processing of the tissue samples. Since there are at present no better methods available to preserve the tissue for histopathological analysis we must confine the discussion to the question whether the alterations observed represent reversible or irreversible events. The astrocytic swelling observed, in itself probably caused by efflux of K^+ from neurons with an ensuing uptake of ions and water by glial cells (for literature and further discussion, see Siesjö 1981), is probably to a substantial degree reversed once the seizure discharge has ceased. There are also reasons to believe that most of the

neuronal alterations are reversible. We base this belief on unpublished results showing considerable regress of the type 1 and type 2 cell changes following interruption of the seizures. Although the present results cannot, therefore, be used to assess the final cell damage incurred, the results allow a comparison of changes observed during status epilepticus in animals in which cerebral oxygen availability was varied, and the results are directly comparable to those reported in a previous communication (Blennow et al. 1978).

As stated in the introduction, the results reported by Blennow et al. (1978) suggested that epileptic brain damage may be "oxidative", e.g. triggered by free radical formation (cf. Siesjö 1981). We chose to study this question by induced hyperoxia (see below), and by manipulating tissue levels of α -tocopherol. Vitamin E is considered an efficient free radical scavenger (Coombs et al. 1975), and lesions in the central nervous system have been reported in vitamin E deficiency (Young et al. 1973, Towfighi et al. 1981). It is also of interest that conditions presumed to cause free radical damage have been reported to cause "consumption" of the lipid-soluble α -tocopherol even when tissue levels of water-soluble scavengers remain constant (see Yoshida et al. 1982). The present results give no indication, though, that the cell damage observed in the present study is influenced by increased tissue oxygen tensions, or by variations in the tissue levels of α -tocopherol. Obviously, it remains to be shown that oxidative or peroxidative mechanisms contribute to the cellular alterations observed in epilepsy.

The results and conclusions of the present study differ from those of the previous study by Blennow et al. (1978). These authors identified by LM microvacuolation and "ischemic" cell change in scattered cortical neurons in the normoxic, normotensive epileptic rats, whereas such changes were less pronounced in hypoxic and moderately hypotensive rats. In the present study, animals with seemingly adequate oxygenation (hyperoxia) had a large proportion of neurons with type 1 and type 2 changes, the neuronal alterations being similar in appearance and extent to those previously observed in normoxic animals (Söderfeldt et al. 1981). Furthermore, we did not find that hypoxia or hypotension reduced the extent of cellular alterations. If anything, superimposed hypoxia or hypotension (MABP 50 mm Hg) appeared to exaggerate the extent of neuronal alterations. Finally, the results

showed that mitochondrial swelling, rarely seen in normoxic or hyperoxic animals, were much more common when status epilepticus was combined with hypoxia or moderate hypotension. Probably, such mitochondrial alterations reflect a more pronounced damage to the neurons.

The differences observed between the present study and that reported by Blennow et al. (1978) can hardly be explained in terms of differences in the experimental procedures. Thus, the animal experiments were performed in the same laboratory, and the physiological variables did not differ. It is, therefore, reasonable to assume that the deviations in results between the two studies are related to differences in morphological techniques or in interpretation of structural alterations.

Clearly, the present results give no support to the view that the epileptic brain damage is oxidative in the sense that its severity is related to the oxygen supply. Rather, the results suggest that hypoxia and "severe" hypotension of the present degrees accentuate the damage. This conclusion is supported both by cell counts, by qualitative examination of the tissue in LM, and by the mitochondrial changes observed in EM.

The present study also revealed that the nuclei of astrocytes became enlarged with clumping of chromatin when status epilepticus was combined with hypoxia or moderate hypotension. Astrocytic nuclei of this character are also present in certain forms of ischemia (Kalimo et al. 1981). The pathogenesis and significance of this glial response are unknown, but it is known that the lactic acid content of the cerebral cortex is considerably increased when status epilepticus is combined with hypoxia or hypotension (Blennow et al. 1978). Since this reaction is not present in uncomplicated status epilepticus (Söderfeldt et al. 1981), in which accumulation of lactic acid is less pronounced, it may in some way be related to the degree of acidosis attained.

As alluded to in the introduction, it has been debated to what extent brain damage due to status epilepticus - both in patients and in experimental animals - depends on complicating hypoxia/ischemia (Norman 1964, Meldrum and Brierley 1973, McNamara 1980, Kreisman 1981). Regrettably, the present results do not provide an unequivocal answer to this question. Thus, although our results favour the idea that cellular alterations during status epilepticus appear even if

cerebral oxygen supply seems adequate, they give no information on the final cell damage incurred. The possibility cannot be excluded, therefore that cellular alterations observed during status epilepticus in adequately oxygenated animals are largely reversible, while these observed with complicating hypoxia or hypotension, even if moderate, "mature" in the recovery period. This possibility receives support from studies with transient ischemia which show that "maturation" of neuronal cell damage may require hours and days to occur (Ito et al. 1975, Klatzo 1979, Pulsinelli et al. 1982). It seems highly justified, therefore, that experiments are carried out in which long recovery periods are allowed to follow histopathological examination is carried out.

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BICUCULLINE-INDUCED EPILEPTIC BRAIN INJURY

Transient and persistent cell changes in rat cerebral cortex in the early recovery period.

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SUMMARY

As shown in a previous study (Söderfeldt et al. 1981), bicuculline-induced status epilepticus of 1 and 2 h duration in ventilated rats gives rise to a profound astrocytic edema in cortical layer 3, as well as nerve cell alterations termed type 1 and 2 injury. Type 1 injured neurons, particularly abundant in layer 3, were characterized by condensation of cell cytoplasm (type 1 a). In some cells the changes were so pronounced that they could not easily be distinguished from each other (type 1 b). Type 2 injured neurons, which were most frequent in layers 5 and 6 were swollen and contained large cytoplasmic vacuoles due to dilation of their rough endoplasmic reticulum.

In the present study, we explored to what extent interruption of the seizure activity reverses the changes observed. To that end, status epilepticus of 1 and 2 h duration was induced by bicuculline before the seizures were arrested by i.v. injection of diazepam. The brain was then fixed by vascular perfusion either 5 min (1 h of seizures) or 2 h (1 and 2 h of seizures) of recovery and cerebral cortical tissue was studied by light (LM) and electron microscopy (EM).

Already 5 min following the arrest of seizure activity most of the astrocytic edema had disappeared, the number of type 1 injured neurons was clearly reduced and type 2 injured neurons had almost completely disappeared. After 2 h of recovery, following 1 h of status epilepticus the edema was virtually absent, few type 1 and no type 2 injured cells were found and the type 1 nerve cell change affected only about 1 % of the neuronal population. When recovery was instituted after 2 h of status epilepticus, numerous dark, triangular neurons were found. In the last group an adequate blood pressure could not be obtained. Therefore, the cellular alterations observed were probably not the result of the seizure activity *per se*.

After 5 min of recovery, EM studies showed type 1 injured cells, similar to those noted in non-recovery animals. However, an increased incidence of swollen mitochondria was observed. After 2 h of recovery the few dark type 1 b injured neurons appeared as severely damaged cells with loosening of cell structure.

INTRODUCTION

In order to characterize structural alterations in the brain caused by sustained epileptic activity we have started a series of investigations, using a model of bicuculline-induced seizures in paralyzed and artificially ventilated rats (Söderfeldt et al. 1981, 1982, Atillo et al. 1982). In all experiments so far performed, histopathological changes were assessed by high-resolution light microscopy and by electron microscopy on tissue fixed by vascular perfusion with glutaraldehyde at the end of seizure period of either 1 or 2 h. Apart from laminar astrocytic edema, two types of nerve cell changes were present (called type 1 and 2 injury). Type 1 injured neurons were shrunken with a condensed, darkly staining cytoplasm (type 1 a), often of such a degree that the nuclear outline could not be discerned in LM pictures (type 1 b). The type 2 cells were characterized by swollen perikarya with cytoplasmic vacuoles due to widened endoplasmic reticulum.

The cell injury observed was surprisingly extensive. Thus, after 1 and 2 h of status epilepticus only 49 and 36 % of neurons, respectively, could be classified as normal (Söderfeldt et al. 1981). The results differ profoundly from those reported by Brown et al. (1980) who found little evidence of damage to hippocampal pyramidal cells after several hours of bicuculline-induced seizures in cats. In contrast to our study their animals were sacrificed after 1-5 h of recovery, following pharmacological interruption of seizure activity. It should also be noted that Meldrum et al. (1973) found unequivocal signs of "ischemic" cell damage in the baboon following seizures induced by bicuculline. The only animal that was allowed a significant recovery period showed very slight neuronal damage, confined to the cerebral cortex, in spite of a seizure duration exceeding 7 h.

Two factors must be taken into account when these discordant results are considered. First, it seems possible that cell damage results, not from the seizure activity per se, but from complicating arterial hypoxia and/or hypotension. Some evidence that this may be the case was presented in a preceeding communication (Söderfeldt et al. 1981 b). Second, the possibility must be taken into account that the cellular alterations observed during seizure activity are largely reversible if the seizure activity is arrested, and the tissue is

fixed by vascular perfusion after some period of recovery.

In order to shed further light on these problems, we induced seizures of 1 h duration by bicuculline in well oxygenated animals, and fixed the tissue by perfusion 5 min or 2 h after interruption of seizure activity by i.v. injection of diazepam. In another group the seizures were allowed to continue for 2 h before they were arrested. However, since blood pressure in these animals could not be maintained in the recovery period, the histopathological study was confined to LM analysis.

MATERIALS AND METHODS

Most of the techniques used in the present study have been described in detail in a previous communication (Söderfeldt et al. 1981). In the following, we therefore give a relatively brief outline of materials and methods.

Animals and seizure model. Male Wistar rats (290-360 g) had free access to water and food pellets until anesthesia was induced with halothane. They were then tracheotomized, connected to a respirator and paralysed with intravenous (i.v.) tubocurarine chloride. After cannulation of the femoral arteries and one femoral vein, the halothane was withdrawn and the animals were ventilated with a mixture of N₂O and O₂ (75:25) for 30 min before the experiment was started. Arterial blood pressure and electroencephalogram (from gold-plated bolts inserted in the skull) were recorded polygraphically. Rectal temperature was maintained close to 37° C by external heating. Prior to bicuculline injection, arterial blood pressure was reduced from 150-160 mm Hg to 100-120 mm Hg by arterial bleeding. This was done to diminish the severity of the early ictal hypertension. Bicuculline hydrochloride (Sigma Chemical Co, St. Louis, MO., USA) was then injected i.v. in a dose of 1.2 mg/kg⁻¹ body weight. For the 1 h or 2 h seizure groups, N₂O in the gas mixture was changed to N₂ during the seizures.

After a seizure period of 1 or 2 h, diazepam was given i.v. The injections were given until the EEG seizure activity had stopped, which took 30 to 180 sec. The amount given varied between 3.5 mg and 7.5 mg per kg body weight. Some animals required additional amounts of

diazepam to prevent reoccurrence of epileptic discharge in the EEG (see below). Four control animals were similarly kept on the respirator for 1 h, then given 5 mg/kg⁻¹ of diazepam and maintained artificially ventilated for another 2 h before fixation.

Sampling and morphological methods. For the fixation of the brain a rapid thoracotomy was performed and a cannula was inserted into the ascending aorta via the left ventricle. The descending aorta was clamped and the right auricle opened. The cerebral vasculature was first rinsed with a 0.9 % NaCl solution (37°C) for about 1 min, followed by 300 ml of 3 % glutaraldehyde (GA) (Polaron Equipment Ltd, Watford, England) in 0.1 mol. l⁻¹ -phosphate buffer, pH 7.4 at 37°C and a pressure of 120 mm Hg. After perfusion, the brain was left in situ for at least 45 min; it was then removed and stored in the same fixative until processed for microscopy. Efficiency of fixation was evaluated by absence of blood in vessels.

One hemisphere of the brain was coronally sectioned and embedded in a plastic (EFL -67, Serva Feinbiochemica, Heidelberg, FRG). From the other hemisphere samples were taken for EM and for embedding in paraffin.

The cortical tissue blocks for EM (Fig. 1) were sectioned into 230 μ m slices with a Sorvall TC-2 tissue sectioner, postfixed in cacodylate buffered 1 % osmium tetroxide, en bloc stained with 0.5 % uranyl acetate in veronal acetate buffer, dehydrated in a graded series of ethanol and embedded in epon. Semithin sections were stained with toluidine blue. They were used for high-resolution LM and selection of areas for thin sectioning. The thin sections were double stained with uranyl acetate and lead citrate and examined in a Zeiss EM 10 or a JEM 100 C electron microscope.

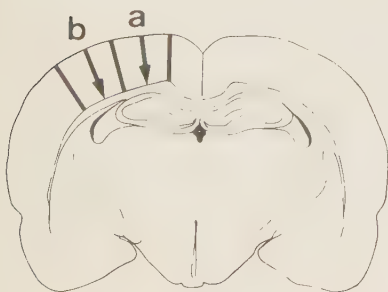


Fig.1 Samples a and b for high-resolution LM and for EM were taken from the fronto-parietal cerebral cortex. Cell counting was performed in 250 μ m wide strips perpendicular to the surface as indicated by the arrows.

In order to quantify the neuronal damage the number of altered cells was determined by counting the neurons in 250 μ m wide strips extending perpendicularly from the surface to the white matter. Each strip contained 70-100 neurons which were classified according to the criteria presented in our first paper (Söderfeldt et al. 1981).

RESULTS

Physiological variables. Changes in arterial blood pressure, blood gases and pH during the seizure period were the same as described in earlier studies on the same model (Meldrum and Nilsson 1976, Chapman et al. 1977, Blennow et al. 1978, Söderfeldt et al. 1981). The mean arterial blood pressure (MABP) rose with the onset of EEG discharge to about 200 Hg. Five to 15 min later, blood pressure slowly declined. After 30 min, infusion of the withdrawn blood and, later of donor blood was needed to maintain blood pressure above 100 mm Hg. After injection of diazepam there was a further tendency to hypotension, which was only partly affected by injections of additional donor blood. Animals in the 1 h epilepsy group could, however, be maintained at MABP level of 100 mm Hg but in the 2 h epilepsy group, there was a decrease of MABP to mean value of 78 mm Hg. In the latter group the arterial pO_2 value also declined.

Mean values for physiological variables recorded just before diazepam injection and at the end of the experiment are given in Table 1.

EEG changes. The onset of seizures was characterized by rapid, irregular spikes, followed by spikes of greater amplitude and rhythmicity (Fig.2). This pattern was then changed into one with bursts of rapid, irregular spikes lasting 1-30 sec separated by periods of EEG flattening of 1-10 sec duration. When diazepam was injected, the EEG spikes slowly declined and the EEG became isoelectric. After a period of 60 min EEG activity returned. Single spikes returned in some animals. When this pattern was seen extra diazepam was given. Animals which showed ictal EEG changes in spite of extra diazepam doses (up to 3.5 mg/kg⁻¹) were excluded from the study.

Table 1. Physiological parameters in the different control and experimental groups.

Group	Mean arterial pressure (mmHg)			Arterial pO ₂ (mmHg)			Arterial pCO ₂ (mmHg)			Arterial pH		
	a	b	c	a	b	c	a	b	c	a	b	c
Diazepam only n = 4	166.5± 5.7	170.0± 7.4	166.5± 7.0	120.2± 5.5	120.6± 2.5	114.4± 1.6	38.0± 2.6	28.0± 3.7	36.7± 1.8	7.44± 0.07	7.43± 0.02	7.44± 0.01
St.ep 1h + recov. 5' n = 7	120.0± 5.9	114.3± 3.9	113.4± 7.6	129.0± 4.5	132.2± 5.6	115.8± 5.7	25.7± 3.9	35.4± 2.9	42.3± 2.6	7.11± 0.12	6.94± 0.12	6.84± 0.13
St.ep 1h + recov. 2h n = 6	116.7± 7.14	142.0± 6.47	100.0± 5.81	132.9± 5.38	130.4± 5.27	124.3± 2.92	20.0± 1.18	32.5± 2.33	37.9± 1.86	7.16± 0.027	6.90± 0.014	7.06± 0.050
St.ep 2h + recov. 2h n = 6	110.8± 3.96	100.0± 3.63	77.5± 4.96	119.2± 2.16	104.9± 1.95	96.5± 4.47	27.9± 1.86	38.3± 1.60	39.5± 1.19	7.06± 0.037	7.02± 0.034	7.08± 0.026

a = after 30 min.

b = just before diazepam, inj.

c = at end of experiment.

Values are means ± S.E.M. of the mean.

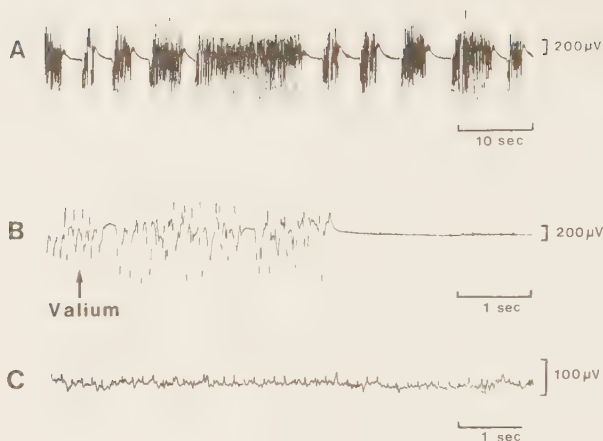


Fig.2 Changes in EEG activity. A. 30 min after injection of bicuculline. B. At injection of diazepam. C. 90 min after injection of diazepam.

MORPHOLOGY

In order to demonstrate any transient and persistent changes in the cerebral cortex we relied on the findings previously described from our laboratories concerning the effects of bicuculline induced status epilepticus for 1 and 2 h without any survival period after the end of the seizures (Söderfeldt et al. 1981). By LM such rats showed a bandformed edema particularly of cortical layer 3 due to swollen astrocytes. Nerve cell changes of different kinds were particularly abundant in the edematous layer 3. Such cells were characterized by a condensation of both karyo- and cytoplasm with marked shrinkage of the cell body. Type 2 injured neurons were fewer in number and were most often seen in cortical layers 5 and 6. They appeared swollen and showed slit-formed cytoplasmic vacuoles chiefly due to swelling of the endoplasmic reticulum. With this information available and since the controls given diazepam appeared completely normal we had a chance to determine to what extent signs of recovery occurred after the seizures had ended. Since we could not exclude the possibility that animals with seizures for 2 h got secondary lesions due to impaired circu-

lation in the postepileptic period the present EM study relies on observations in rat with status epilepticus for 1 h followed by a recovery period of 5 min or 2 h.

Macroscopically the brains from the recovery animals looked normal and by LM there was most often no blood in the vessels. Fixation was therefore considered as adequate.

Status epilepticus for 1 h followed by recovery for 5 min.

By LM the cortex showed changes of mainly the same type and distribution as earlier described in animals perfused during seizure activity (Söderfeldt et al. 1981). However, cell counts (Table 2) revealed that 74 per cent of the neurons were normal or with only minor changes compared with 49 per cent in animals sustaining status epilepticus for 1 h without recovery (Söderfeldt et al. 1981).

Among the abnormal cells the type 1 injured neurons were seen only in layer 3 (Fig. 3). Rounded intra-cytoplasmic vacuoles were more common in these cells than in non-recovery animals (Söderfeldt et al. 1981). The type 2 change (Fig. 4) had almost entirely disappeared and was now only 1 per cent compared with 9 per cent in non-recovery animals (Söderfeldt et al. 1981).

Table 2

Relative proportions of normal and different types of injured neurons.

Group	Normal or minor changes	1a	1b	2
St ep 1 h + recovery 5'	74.0 % + 4.1	21.0 % ± 3.9	3.0 % ± 1.0	1.0 % ± 0.8
St ep 1 h + recovery 2 h	98.5 % ± 0.6	0.9 % ± 0.3	0.4 % ± 0.2	0

Values are mean ± S.E.M. of the mean

EM revealed that the neuronal alterations were even at the subcellular level of the same type as in non-recovery animals (Fig. 5). The only exception was that mitochondrial swelling, which was only rarely seen in our earlier study, occurred more frequently in this group. Thus some of the vacuoles in the dark condensed type 1 neurons originated from dilated mitochondria (Fig.6).

By LM cortical layer 3 was edematous (Fig. 3) but not to that extent as shown in our previous study on non-recovery animals (Söderfeldt et al. 1981). By EM it was shown that the remaining edema was located in swollen astrocytic processes around vessels and some neurons.

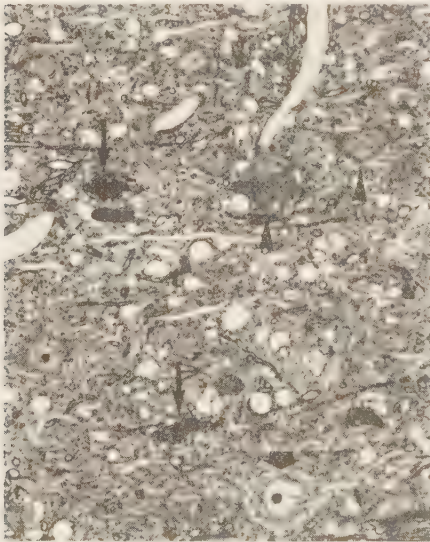


Fig.3

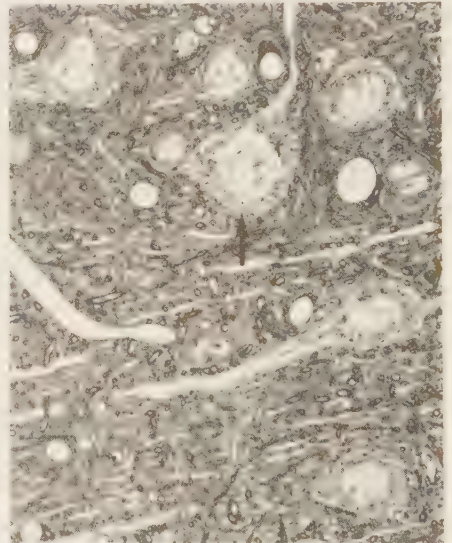


Fig.4

Fig.3 After 5 min of recovery following 1 h of epilepsy the tissue in cortical layer 3 is still somewhat edematous though less than in the non-recovery animals. There are a couple of type 1a (arrowheads) and two type 1b (arrows) injured neurons the upper one of the latter having tiny vacuoles in its cytoplasm (cf. Fig.5). The thick arrow points to a normal appearing neuron. Epon + toluidine blue 730 X.

Fig.4 In the cortical layer 5 of the same rat as in Fig.2 there is a type 2 injured neuron (arrow) with perinuclear and some cytoplasmic vacuoles (cf. Fig.4). The other neurons appear quite normal and the neuropil is not edematous. Epon + toluidine blue 730 X.

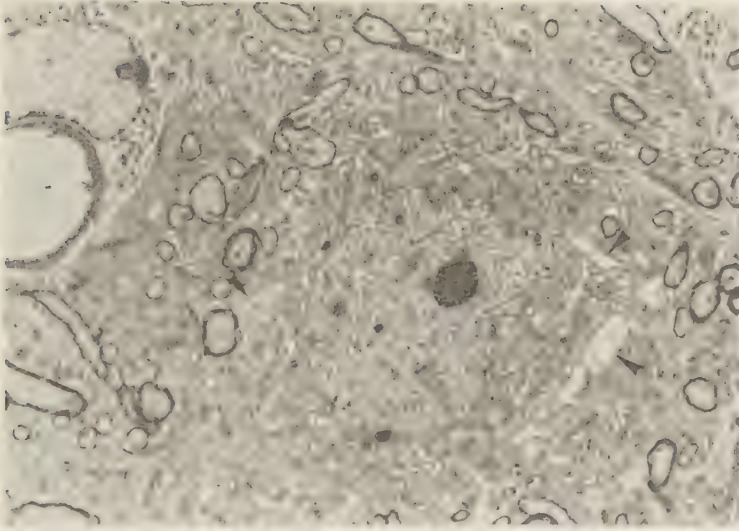


Fig.5 An electron micrograph of a type 2 injured neuron, a few of which were still present after 5 min of recovery following 1h of epilepsy. The cisternae of the endoplasmic vacuoles are dilated, while otherwise the cell appears quite normal. Some perineuronal astrocytic processes show slight edema (arrowheads). 3300 X.

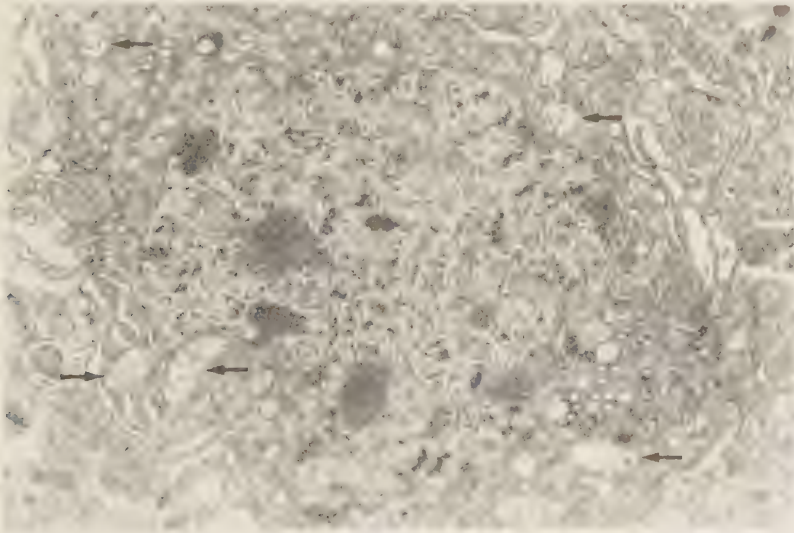


Fig.6 Status epilepticus 1h, recovery 5 min. Injured neuron type 1a from cortical layer 3. Note that some dilated mitochondria are seen. 18000X

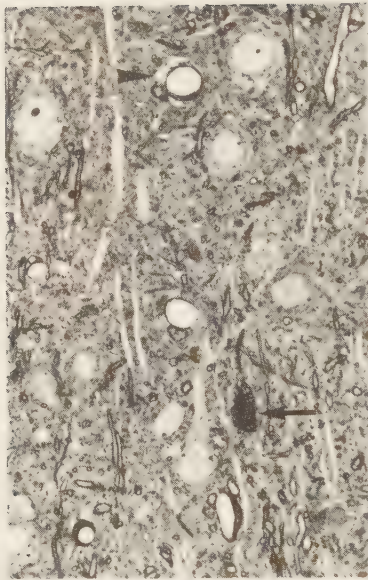


Fig.7

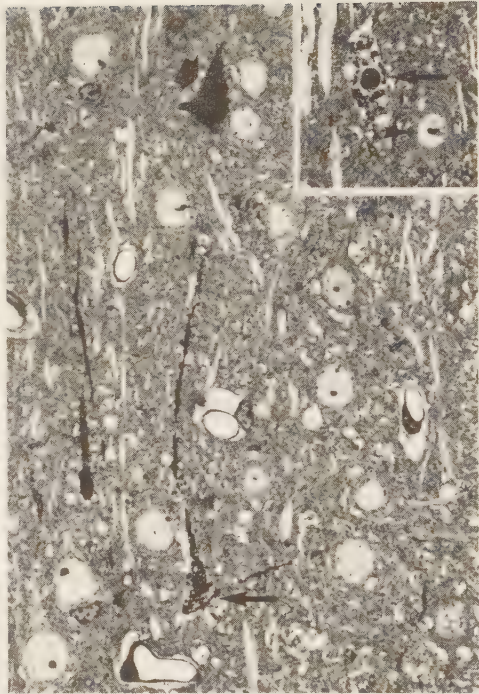


Fig.8

Fig.7 In rats with 1h of epilepsy followed by 2h of recovery most of the edema in cortical layer 3 had disappeared with only minor perivascular vacuoles remaining (arrowhead). One persisting type 1b injured neuron (arrow) is present among the normal appearing ones. Epon + toluidine blue 730 X.

Fig.8 The cortical layer 3 of the rat with most marked residual changes after 2h of recovery following 1h of epilepsy still appears somewhat edematous, most markedly around capillaries. The remaining type 1 injured neurons show progressive darkening (thick arrow) or even fragmentation (arrow). Inset: The cytoplasm around the pyknotic nucleus has broken into dark fragments (arrow). The neighboring cell (arrowhead) is nearly normal. Epon + toluidine blue 730 X.

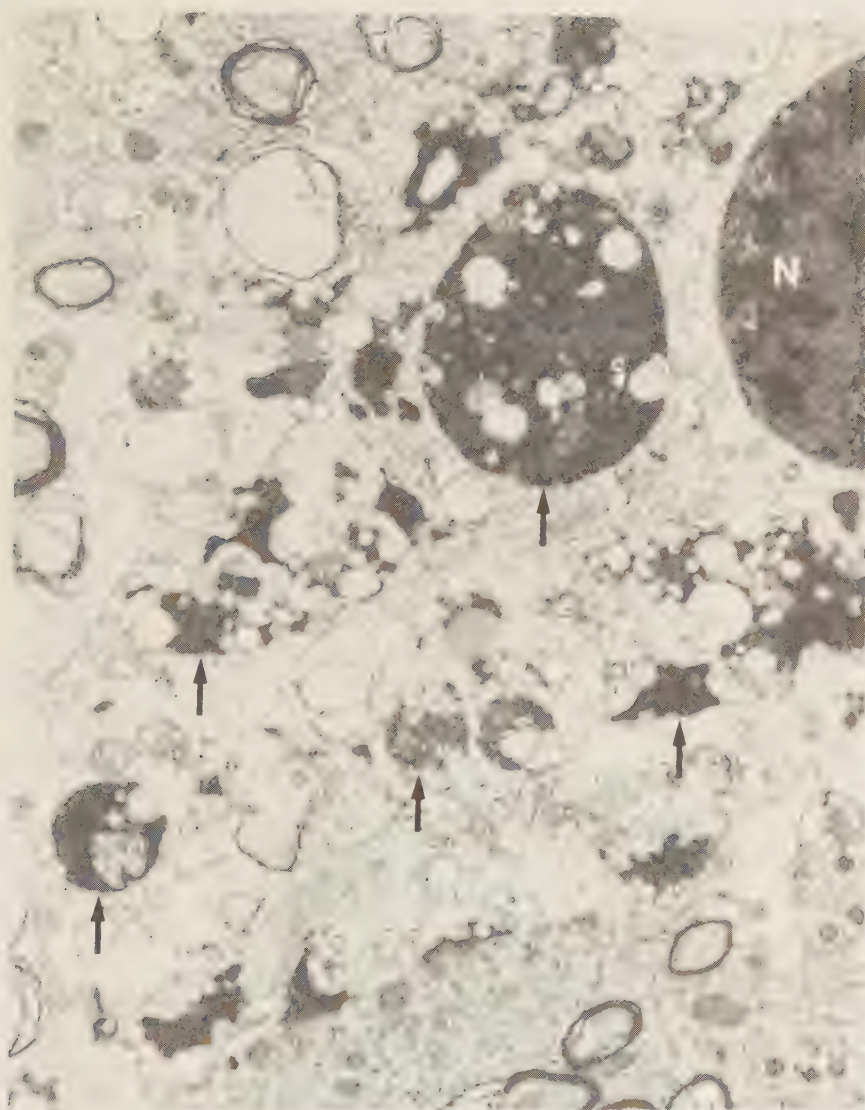


Fig.9 Electron micrograph corresponding to the fragmented cell in the inset of Fig.7. The disrupted cytoplasm contains clumps of dark material of which some are marked with arrows. Nucleus (N) of the neuron is condensed and very electron-dense. 16000X.

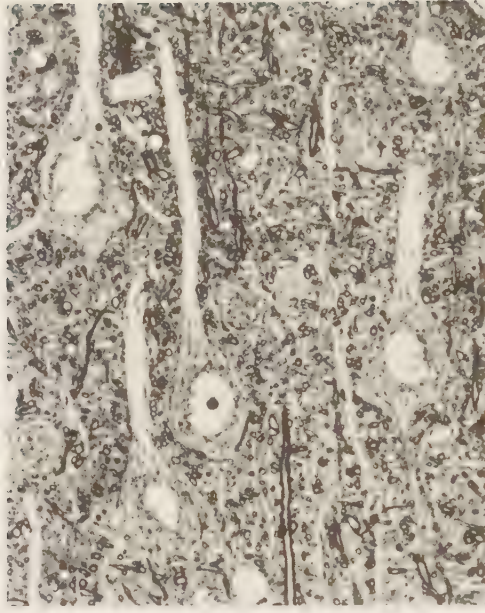


Fig.10 After 2h of recovery following 1h of epilepsy the type 2 injured neurons had disappeared and the tissue in layer 5 appears intact. Epon + toluidine blue 730 X.

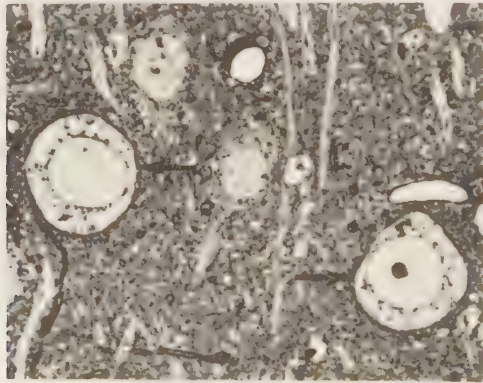


Fig.11 In the group of 2h of epilepsy with 2h of recovery cells (arrows) with clear cytoplasm, centrally located organelles and evenly distributed nuclear chromatin were seen in addition to the characteristic type 1 and 2 changes. Epon + toluidine blue 730 X.

Status epilepticus for 1 h followed by recovery for 2 h.

Compared with the preceding group and non-recovery rats the LM structure of the brain from animals with recovery for 2 h approached normal appearance. However in layer 3 a few neurons (about 1.4 per cent) of type 1 remained (Fig. 7). Some of them had become fragmented indicating progression of the type 1 nerve cell injury (Fig. 8). The ultrastructural appearance of these cells is illustrated in Fig. 9. The type 2 nerve cell injury which was still present after 5 min of recovery had now disappeared (Fig. 10, Table 2).

The resorption of the bandformed edema which had begun at 5 min of recovery was now almost completed. Only a slight perivascular edema remained around the cortical capillaries (Fig. 7).

Status epilepticus for 2 h followed by recovery for 2 h.

As in the previous groups type 1 injured neurons were present in layer 3 of all the rats.. In four of the six animals such cells were also seen in layers 5 and 6. Among this cell group the majority was of type 1 h or showed fragmentation, i.e. signs of more pronounced cell damage. Very few type 2 neurons were seen in only one of the animals.

Finally, a peculiar type of nerve cell injury was seen in three of the six rats. It was characterized by a normal or slightly swollen perikaryon, a pale cytoplasm with centrally located organelles (lysosomes?) and evenly dispersed nuclear chromatin (Fig. 11). These cells bear resemblance to the type II injury in severe hypoglycemia (Agardh et al. 1980). The bandformed edema was less marked than in non-recovery animals (Söderfeldt et al. 1981) but was as expected somewhat more pronounced than in the preceding group.

DISCUSSION

The present results have shown that although 1 h of bicuculline-induced status epilepticus in ventilated and well oxygenated rats leads to extensive astrocytic edema in the cerebral cortex and, at least by conventional histopathologic criteria, to widespread nerve cell alterations (Söderfeldt et al. 1981), virtually all of these changes are quickly reversible when the seizures are arrested and the animals allowed a recovery period before the brain is processed for

histopathologic analysis. In view of this, the difference in results between our previous report (Söderfeldt et al. 1981) and that of Brown et al. (1980) is reduced to the question whether or not a small proportion of neurons (about 1-2 % of the total population) remains permanently damaged. However, it remains to be discussed why prolongation of the seizure period to 2 h was accompanied by signs of more severe neuronal damage in spite of the 2 h recovery period.

The usefulness of pharmacological methods of seizure induction for studies of the relationship between seizure activity and brain cell damage rests on two requirements : (1) the agent used should not be neurotoxic in any other sense than that causing paroxysmal neuronal activity, and (2) it should not lead to systemic alterations that by themselves give rise to structural alterations.

As judged by published reports, some convulsive agents, notably methoxypyridoxine and kainic acid, seem to possess special neurotoxic characteristics. Thus, whereas seizures induced by methoxypyridoxine were found to be associated with extensive damage to hippocampal CA 3 and CA 4 neurons, those elicited by strychnine and metrazol were not (Purpura and Gonzales-Montaguado 1960). Furthermore, the cell damage recorded following kainic acid-induced limbic seizures in rats (Lothman and Collins 1981) appears much more marked than that caused by comparable period of bicuculline-induced seizures (Brown et al. 1980 and present results).

In view of these results, and of the accepted actions of bicuculline as a GABA receptor blocker, it appears less likely that the alkaloid has special neurotoxic actions. In this respect, therefore, the agent should be ideal for studies of the relationship between sustained seizure activity and neuronal cell damage. However, bicuculline does not fulfill the second requirement discussed above since it has profound systemic effects, some of which may well jeopardize the viability of brain cells. For example, the drug-induced seizures lead to an intense sympathoadrenal activation with an increase in blood pressure which quickly causes cardiac failure unless blood pressure is reduced prior to seizure induction and profound systemic acidosis may also develop (Meldrum and Nilsson 1976, Chapman et al. 1977). Furthermore pulmonary edema may be another complication.

One consequence of the systemic effects elicited by bicuculline is that disruption of the blood-brain barrier occurs early in the seizure discharge (Johansson and Nilsson 1977, Persson et al. 1980), another that secondary arterial hypotension ensues, possibly related, at least in part, to the sustained acidosis. We encountered considerable difficulties in assembling a group of recovery animals following 2 h of seizure activity, and even the animals included in the group had unacceptably low mean arterial blood pressures. We submit, therefore, that the results obtained in that group do not reflect cell damage which can be attributed to the 2 h seizure activity per se. Obviously, the model of bicuculline-induced seizures does not lend itself to studies of the long-term neuropathological effects of sustained seizures unless means are found to combat the systemic, particularly the cardiovascular complications. However, the results obtained seem useful in the sense that they emphasize the importance of changes in systemic variables when conclusions are drawn regarding seizure duration and neuronal cell damage.

We put more confidence on the results obtained after 1 h of seizure activity. However in view of the systemic alterations elicited we cannot confidently state that 1 h of status epilepticus caused by other mechanisms will lead to permanent neuronal damage in about 1-2 % of the cells (see Purpura and Gonzales-Montaguado 1960, Brown et al. 1980). Therefore, the most important general result of this study concerns the rapid reversal of those astrocytic and neuronal alterations which are present in non-recovery animals (Söderfeldt et al. 1981). It does not seem difficult to explain the reversibility of the astrocytic edema and of the type 1 a and type 2 injury to neurons. Thus, since astrocytic swelling may merely represent a functional response to release of potassium from nerve cells (for literature, see Siesjö 1981), it seems natural that this swelling resolves when the ionic and water shifts are reversed upon termination of the paroxysmal activity. It also seems possible that such shifts are responsible for the tinctorial changes in the type 1 a injured cells, and the intracellular vacuolization of the type 2 injured cells. However, in view of the extensive condensation of the type 1 b cells (see Söderfeldt et al. 1981) it was surprising that this cell alteration proved largely reversible. Clearly the results demonstrate that great caution must be exercised when inferences on irreversible cell damage are drawn from

tissue specimens collected during an insult that is accompanied by major shifts in electrolytes and water.

The present results lead to the tentative conclusion that bicuculline-induced seizures of 1 h duration lead to permanent damage to a small proportion of neurons in the cerebral cortex. Our parallel studies (Atillo et al. 1983) allow a similar conclusion to be drawn for certain neuronal populations in the hippocampal formation. We wish to emphasize, though, that this conclusion is tentative. Thus, we can neither exclude that prolongation of the recovery period will lead to additional reversal of histopathological alterations nor that such prolongation allows "maturation" of cell damage in neurons that are seemingly normal (cf. Ito et al. 1975, Kirino 1982, Pulsinelli et al. 1982). Obviously, these problems can only be solved through the development of seizure models that permit extended recovery periods to be studied.

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PATHOGENESIS OF BRAIN LESIONS CAUSED BY EXPERIMENTAL EPILEPSY
Light and electron microscopic changes in the rat hippocampus
following bicuculline-induced status epilepticus

A. Attilio, B. Söderfeldt, H. Kalimo, Y. Olsson, and
B.K. Siesjö

Summary.

Status epilepticus with a duration of 1 or 2 h was induced in rats by i.v. injection of the GABA receptor blocking agent, bicuculline. Immediately thereafter, or following a 2 h recovery period, the brains were fixed by vascular perfusion and processed for light and electron microscopy in order to characterize the type and distribution of morphological changes in the hippocampal formation.

In a previous study (Söderfeldt et al. 1981) astrocytic edema and wide-spread neuronal changes of two different kinds occurred in the fronto-parietal cortex of the same animals. Type 1 injured neurons were characterized by condensation of karyo- and cytoplasm (type 1a), which in some neurons became so intense that the nucleus could no longer be clearly discerned (type 1b). The type 2 injured neurons had slit-formed cytoplasmic vacuoles chiefly caused by dilation of the rough endoplasmic reticulum.

In the hippocampus the most conspicuous alteration was astrocytic edema which was most marked around the perikarya of pyramidal neurons in CA 1 to CA 4 and subiculum. In the dentate gyrus the edema was less pronounced and when present affected particularly the hilar zone of the stratum granulosum. The nerve cell changes were less pronounced than in the cerebral cortex. The vast majority of the hippocampal pyramidal neurons in CA 1 - CA 4 showed minor configurational and tinctorial abnormalities (incipient type 1a change). Severe nerve cell alteration (type 1b) were present but very rarely affected the pyramidal neurons of CA 1 - CA 4 and subiculum, whereas in the dentate gyrus pyramidal basket neurons of stratum granulosum and pyramidal nerve cells in stratum polymorpha showed the severe type 1b changes. As compared with the fronto-parietal cortex (Söderfeldt et al. 1981) the type 2 changes were extremely rare. In the early recovery period after 1 h of status epilepticus the astrocytic edema and the incipient type 1a changes had almost entirely disappeared whereas a few condensed and darkstaining, type 1b, injured neurons remained.

Thus, in this model of status epilepticus the most marked response in the hippocampal formation is astrocytic edema in the layers where pyramidal perikarya are located. Incipient, mild nerve cell changes which appear to be reversible were frequent and wide-spread in the entire hippocampal formation.

INTRODUCTION

Observations on human material have revealed that the hippocampus is the area which shows the most constant and pronounced abnormalities in cases with severe epilepsy (Bouchet and Cazauvieilh 1825; Norman 1964; Corsellis and Meldrum 1976). In patients who have died after an attack of status epilepticus swelling is often present particularly in the region of the Sommer sector and the dentate fascia. Nerve cell abnormalities and subsequent gliosis are most pronounced in these areas and in the end folium, whereas neurons lying between these fields are relatively spared, the so-called resistant part (Corsellis and Meldrum 1976; Dam 1980). Gross atrophy and extensive neuronal loss with gliosis, i.e. the "Ammon horn sclerosis", can be found in as many as 50 per cent of patients with chronic epilepsy (Margerison and Corsellis 1966).

Ever since Sommer's observations (1880) the hippocampus has attracted considerable attention in the discussion of the pathogenesis of histopathological changes associated with epilepsy. Based on experience from human material diverging views have been put forward regarding the question whether or not the hippocampal changes are induced by epilepsy. At first they were considered to be present before the epileptic attacks started, and it was then postulated that the hippocampus became damaged by ischemia at birth or by other injuries during childhood. This would then result in the sclerosis which could develop into an epileptogenic focus (Earle et al. 1953; Gastaut 1959). Others had the idea that the neuronal damage in the hippocampus was secondary to the seizures and attributed it to disturbed blood and oxygen supply, i.e. the particularly vulnerable hippocampus might suffer an ischemic or anoxic injury during the epileptic fits (Spielmeyer 1927; Scholz 1951, 1959). Finally, a few investigators considered that a direct relationship does not always exist between hippocampal damage seen in autopsy cases and epilepsy (Morel and Wildi 1956; Haymaker et al. 1958).

Human material can only provide limited information about the pathogenesis of brain changes associated with epilepsy. Several approaches have, therefore, been made to create animal models of drug-induced sustained seizures. In these, agents have been used that stimulate excitatory systems (kainic acid, glutamic acid) or decrease inhibitory activity (Woodbury 1981). Among the latter group the plant

alkaloid bicuculline, which blocks the inhibitory action of GABA (Curtis and Johnson 1974; Heyrer et al. 1981) has been found to induce long-lasting electroencephalographic seizures in baboons, rats and cats (Meldrum et al. 1973; Meldrum and Brierey 1973; Meldrum and Nilsson 1976; Chapman et al. 1977; Brown et al. 1980).

The model with bicuculline-induced epilepsy has been extensively characterized with regard to cerebral energy metabolism and blood flow (Meldrum and Horton 1973; Meldrum and Nilsson 1976; Chapman et al. 1977; Folbergrová et al. 1981; Siesjö et al. 1982; Horton et al. 1980) as well as regional protein synthesis (Kiessling and Kleihues 1981). In some of these studies, changes in tissue metabolites as well as in metabolic rate were measured in both cerebral cortex and hippocampus (Folbergrová et al. 1981; Siesjö et al. 1982; Ingvar and Siesjö, in press). Although the results clearly demonstrate that the seizure-induced metabolic perturbation in the hippocampus is at least as pronounced as in the cerebral cortex, available histopathological results do not give evidence of extensive damage to hippocampal neurons.

Light microscopically (LM), bilateral ischemic nerve cell changes have been found in the hippocampus of baboons (Meldrum and Brierley 1973) and rats (Blennow et al. 1978) particularly in Sommer sector and to a lesser degree in the end folium. However, such changes involved only a few neurons, and in a cat model with seizures for as long as 5 h no LM - and electromicroscopical (EM) abnormalities could be detected in the hippocampus (Brown et al. 1980).

We have started to investigate the reaction of rat brain parenchyma to sustained epileptic seizures induced by systemic injection of bicuculline, using high resolution LM and EM methods. Status epilepticus for 1 or 2 h caused changes in the cerebral cortex characterized by astrocytic edema and nerve cell abnormalities of two kinds (Söderfeldt et al. 1981). One cell type showed vacuolization in the perikaryon chiefly due to dilatation of the endoplasmic reticulum and the other shrinkage and hyperchromasia in the absence of marked mitochondrial abnormalities. In view of the importance of hippocampal changes in chronic epilepsy we have now extended our LM and EM observations to include various subregions of the hippocampus in the rat model of bicuculline-induced status epilepticus.

MATERIAL AND METHODS

The material derives from animals used in previous studies on the LM and EM changes in rat cerebral cortex. In these studies, the brains were perfusion-fixed either immediately after bicuculline-induced status epilepticus of 1 or 2 h duration (Söderfeldt et al. 1981) or after recovery period of 5 min or 2 h following 1 h of continuous seizures (Söderfeldt et al., in preparation). The model will only be briefly described.

Animals and seizure model

Male Wistar rats (290-350 g) were anesthetized with halothane, a tracheostomy was performed and the animals were immobilized with tubocurarine chloride. They were then artificially ventilated on a N₂O - halothane-oxygen mixture. Rectal temperature was kept close to 37°C. A femoral artery was cannulated for continuous blood pressure recording and for blood sampling. EEG was recorded from gold-plated bolts inserted in the skull bone.

Following the end of the operative procedure and the discontinuation of halothane supply, the rats were maintained at a steady state for 15-20 min. The blood pressure was then reduced to 100 mm Hg by withdrawal of arterial blood, and bicuculline hydrochloride (Sigma Chemical Co.) was injected i.v. in a dose of 1.2 mg.kg⁻¹ body weight. During the seizures oxygen supply was adjusted to keep arterial PO₂ above 100 mm Hg, and mean arterial blood pressure was maintained above 110 mm Hg by infusion of heparinized blood. In order to study changes in the early recovery period, groups of rats received i.v. diazepam to arrest seizure activity (Söderfeldt et al. 1983).

Sampling. The experimental animals were killed by vascular perfusion of their brains. A detailed description of this procedure and of the fixatives used are presented in a previous paper (Söderfeldt et al. 1981). Briefly, a 3% glutaraldehyde fixative in 0.10 or 0.15 mol.l⁻¹ phosphate buffer, pH 7.4, 37°C, was used for most experiments. The number of animals in the different groups is given in Table 1 (see also Söderfeldt et al., in preparation).

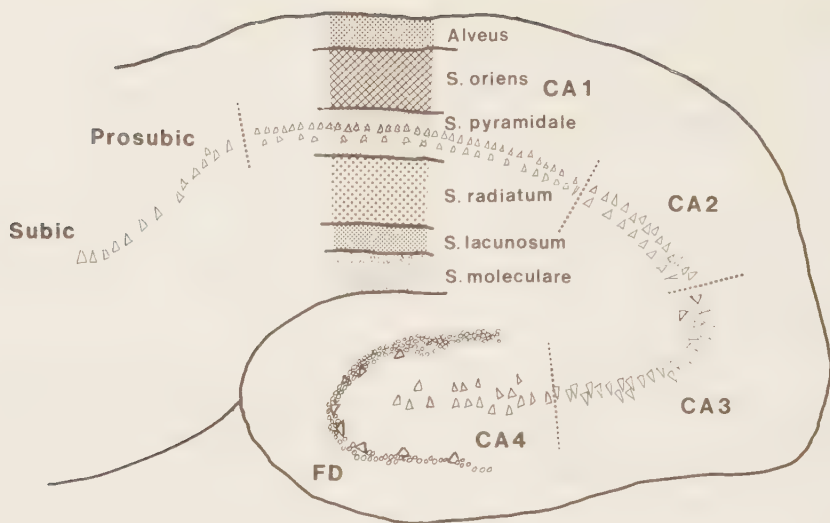


Fig. 1. Schematic presentation of the hippocampal formation in the rat seen in a coronal section. Area dentata contains the layers of granule neurons in the fascia dentata (FD). In its hilar region CA₄ neurons are enclosed. Sommer sector corresponds roughly to the CA₁ region of Lorente de Nô. Subiculum is separated from CA₁ by presubiculum.

The brains were cut in 2-2.5 mm thick coronal blocks. From one of the hemispheres (about 6 mm from the occipital pole) two samples were taken from the hippocampal region. One medial sample contained dentate gyrus, parts of CA 1, CA 4 and subiculum the other mainly CA 2 and CA 3 (Fig. 1). From each sample four coronal slices, 230 microns in thickness, were cut with a Sorvall TC-2 tissue sectioner, processed for EM with embedding in semithin epon sections, and were then stained with toluidine blue for high resolution LM. Thin sections for EM were taken from the tip of the dentate fascia, CA 3

and from the middle part of the Sommer sector in CA 1 b. EM was restricted to rats taken immediately after the seizure periods. From other coronal blocks the entire hemisphere was embedded in plastic (JB-4, Polysciences Inc., Warrington, Pa, USA or EFL-67, Serva Feinbiochemica, Heidelberg, Germany). The were stained with hematoxylin-eosin and with toluidine blue.

Cell counts. In order to get an idea about possible recovery of cell changes and possible regional variations in the pathological alterations 100 neurons in each of the various CA regions, dentate gyrus and subiculum were examined by LM (epon sections). They were classified in the same way as described in our previous paper (Söderfeldt et al. 1981). Type 1 injured neurons were darkly stained, shrunken cells which could be divided into two subpopulations, a and b, depending on the possibility to identify the nucleus (a) or not (b). Type 2 injured neurons contained several easily visible cytoplasmic vacuoles in the absence of shrinkage and hyperchromasia.

Nomenclature. No unitarily accepted nomenclature of the various regions in the hippocampal formation exists and it is also difficult to delineate the various areas from each other precisely. In this paper we have followed Lorente de Nó's CA nomenclature (1934) but, for comparison with other investigators, we also refer to the terminology h₁-h₅ used by Rose (1926) and that by Doinikow (1909). A schematic presentation of the hippocampal formation is given in Fig. 1. Definitions of the various hippocampal regions of the rat brain have previously been presented by Blackstad (1956) and by Raisman et al. (1965). The frequently used term "Sommer sector" corresponds approximately to the CA 1 area of Lorente de Nó and the h₁ area of Rose.

RESULTS AND COMMENTS

GENERAL OBSERVATIONS

As described previously, the onset of seizure discharge was characterized by rapid, irregular spikes, followed by spikes of greater amplitude and rhythmicity. This pattern was then changed into one with bursts of rapid irregular spikes, lasting 1-30 sec, separated by periods of EEG flattening of 1-10 sec duration, a pattern which prevailed for the duration of seizure activity (see Meldrum and Nilsson 1976; Chapman et al. 1977; Söderfeldt et al. 1981). When

Table 1. Physiological parameters in the different status epilepticus groups.

	Arterial pressure mm Hg			Arterial pO ₂ mm Hg			Arterial pCO ₂ mm Hg			Arterial pH		
	a	b	c	a	b	c	a	b	c	a	b	c
Status epilepticus 2 h without recovery n=7	124 + 5 —	116 + 5 —	—	135 + 6 —	111 + 8 —	—	28 +2.0 —	44 +2.1 —	—	7.015 +0.015 —	7.005 +0.062 —	—
Status epilepticus 1 h without recovery n=4	128 + 6 —	115 + 2 —	—	131 + 3 —	122 + 2 —	—	27 +2.6 —	39 +2.0 —	—	7.089 +0.063 —	7.007 +0.011 —	—
Status epilepticus 1 h with recovery 2 h n=5	117 + 7 —	142 + 6 —	100 + 5 —	132 + 5 —	130 + 5 —	124 + 3 —	20 +1.1 —	32 +2.3 —	38 +1.3 —	7.165 +0.027 —	6.906 +0.014 —	7.060 +0.050 —

The values are + S.E.M.

a. After 30 min of EEG seizures

b. At the end of the seizure period

c. After 2 h of recovery.

diazepam was injected after a seizure period of 1 h, the EEG spikes slowly disappeared and the EEG transiently became isoelectric; later slow wave activity appeared (Söderfeldt et al. 1983).

Physiological variables for the present groups have been given in Table 1. Following the ictal increase in blood pressure (to 180–215 mm Hg) it slowly declined. However, by infusion of blood the pressure could be maintained above 100 mm Hg. Arterial PO_2 was well above 100 mm Hg. Arterial PCO_2 fell (from control values of 35–40 mm Hg) early during seizures but then returned to values of about 40 mm Hg. The seizures were associated with marked plasma acidosis. In animals in which the seizures were arrested by diazepam blood gas and pH values were similar. There was a tendency to further fall in blood pressure; however, the pressure could be maintained above 100 mm Hg in all the animals included in the study.

In control animals, the hippocampus had the same structural appearance as previously described in normal rats (Blackstad 1956, 1963; Westrum and Blackstad 1962; Ribak and Andersson 1980; Laurberg and Zimmer 1981). Rats subjected to status epilepticus for 1 or 2 h all showed clearcut morphological changes. The major LM abnormality in the epileptic rats was status spongiosus, i.e. sign of edema, throughout the pyramidal cell layer in CA 1 – CA 4, subiculum and the hilar region of the stratum granulosum in the dentate gyrus. By LM nerve cell changes were also seen throughout CA 1 – CA 4 and to a lesser degree in the dentate gyrus but, in general, they were not at all so pronounced as in the fronto-parietal cortex previously described (Söderfeldt et al. 1981). In LM the neurons were somewhat more irregular in outline and more darkly stained than in the controls (incipient type 1 a changes). Condensed neurons of the kind (type 1 b) seen in the fronto-parietal cortex (Söderfeldt et al. 1981) were very rare. The same was true for the vacuolated type 2 injured neurons. In the animals allowed to recover the status spongiosus and the incipient type 1 a nerve cell changes were almost entirely reversed whereas a few type 1 b injured neurons remained.

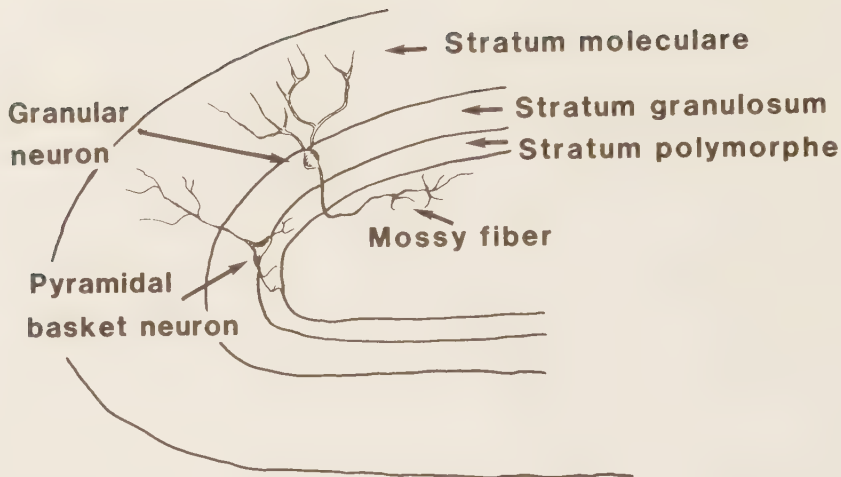


Fig. 2. Schematic presentation of the area dentata. The granular neurons of the stratum granulosum have apical dendrites oriented towards the pial surface. Their axon forms the so-called mossy fibres of CA₄ and CA₃.

AREA DENTATA

NORMAL. As an introduction to more detailed LM and EM descriptions of the pathological findings and account of the various layers in the dentate gyrus and their morphological features of normal rats will first be given.

The dentate gyrus consists of three distinct layers (Fig. 2). The outer one, called stratum moleculare (Raisman et al. 1965), contains the peripheral dendrites of the granule cells, a few astrocytes, and blood vessels. There is, therefore, an abundance of closely packed dendrites, which on the basis of synaptic connections may be divided into a peripheral and a central zone. It is known that the two zones may respond differently towards pathological influences (Kirino and Sano 1980).

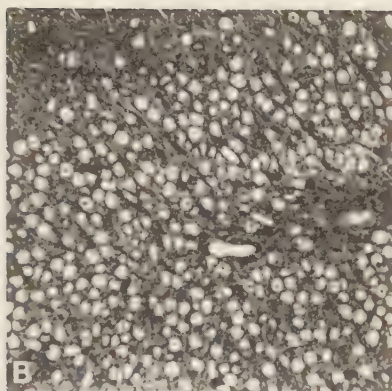
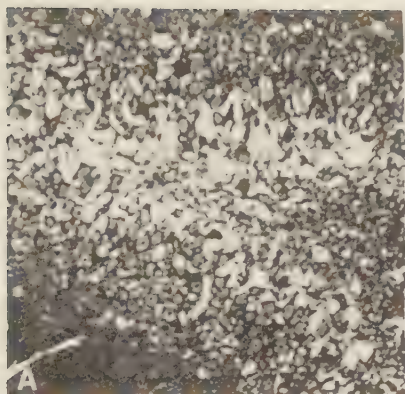


Fig. 3. LM appearance of the area dentata in a rat with status epilepticus for 2 h (a) and after a recovery period for 2 h (b). Note the presence of edema around the nerve cell bodies, the abnormal configuration of neurons and the type 1 b neuron in a and the virtually complete reversal of edema in b.

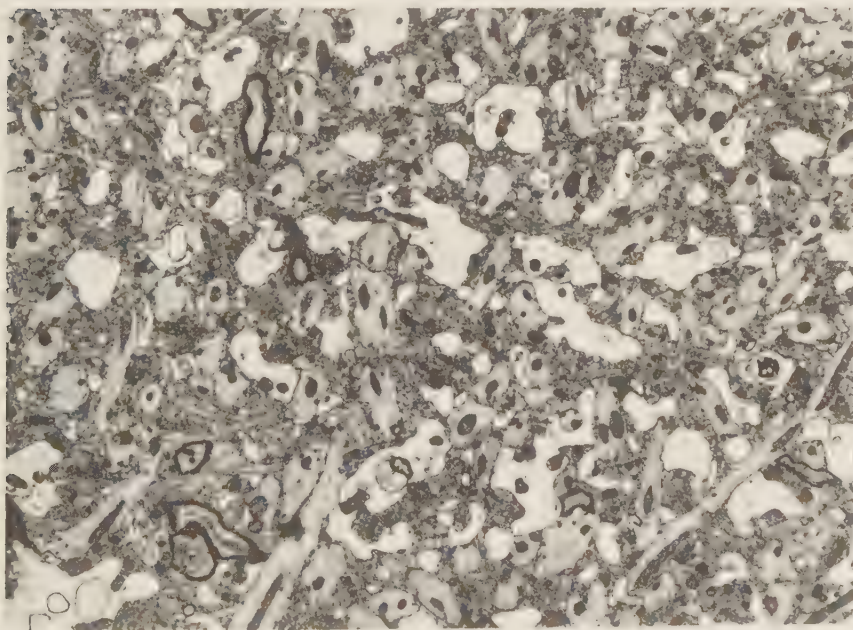


Fig. 4. Stratum moleculare from the dentate gyrus in a rat with status epilepticus for 2 h. Note the widespread astrocytic edema in the form of clear processes (A) and the absence of dendritic alterations. Magn. 3200X.

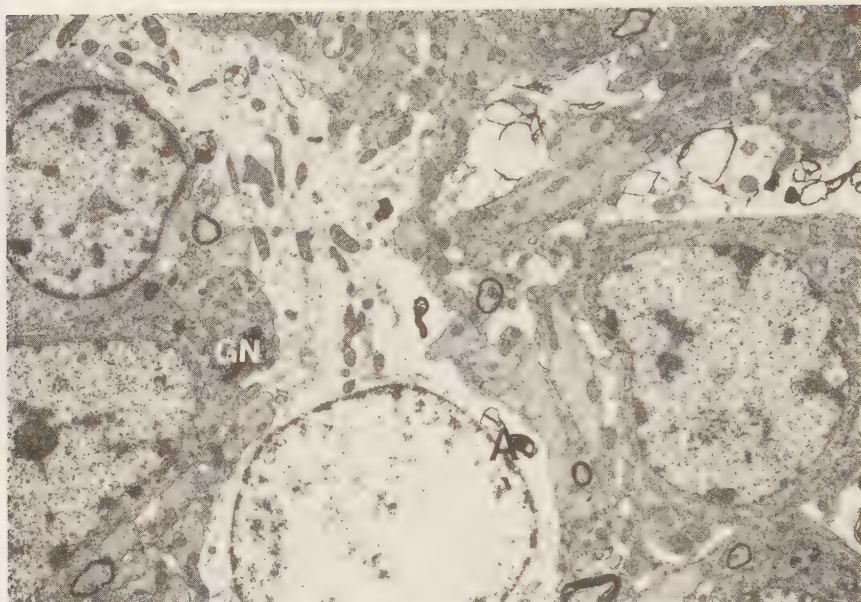


Fig. 5. Stratum granulare in the dentate gyrus from a rat with status epilepticus for 2 h. There is one swollen astrocyte (A) with a watery cytoplasm extending between the neurons. Adjacent granular neurons (GN) and dendrites have an almost normal appearance. Magn. 2600X.

The middle layer, stratum granulosum, consists of densely packed granule cells, i.e. neurons with a rounded configuration, scarce cytoplasm and multiple dendrites originating directly from the perikaryon without any apical dendritic shaft. Their axons pass through the stratum polymorphe (mossy fibres) to make synaptic contacts with neurons in CA 3 and CA 4 (Raisman et al. 1965; Laurberg and Zimmer 1981). The middle layer also contains a few so-called pyramidal basket neurons (Seress 1978) of a larger size with a greater number of rough endoplasmic reticulum and infolded nuclear membrane; cells which probably are GABA-ergic and provide inhibition of other neurons (Ribak and Anderson 1980). Finally, there are some astrocytes but, as seen by EM, fewer glial processes occur between the granule cell somata than in most other areas of the brain (Seress 1978).

The innermost layer, stratum polymorphe, is not clearly separated from CA 4 and is heterogenous in composition. There is a

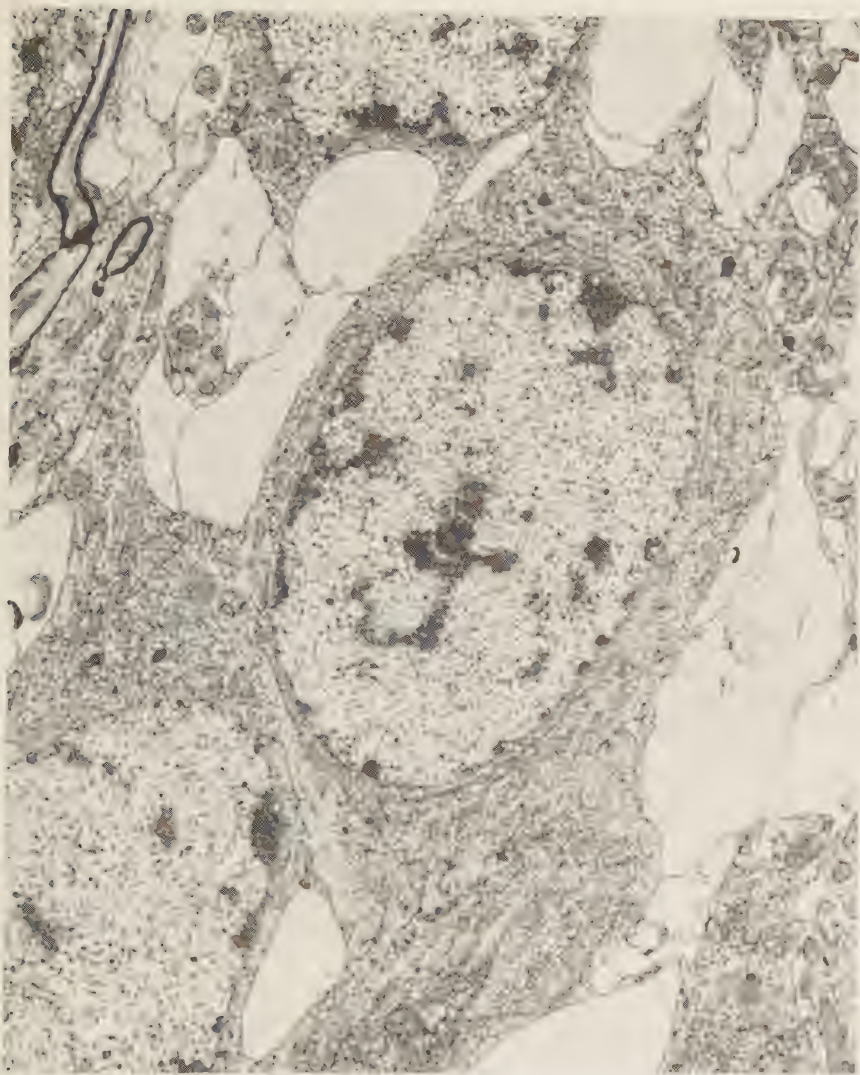


Fig. 6. Stratum granulare in the dentate gyrus from the same rat as shown in Fig. 5. The cellular outline of the granular neuron in the center is somewhat more irregular than normal apparently due to the swollen astrocytic processes close to the neuron.

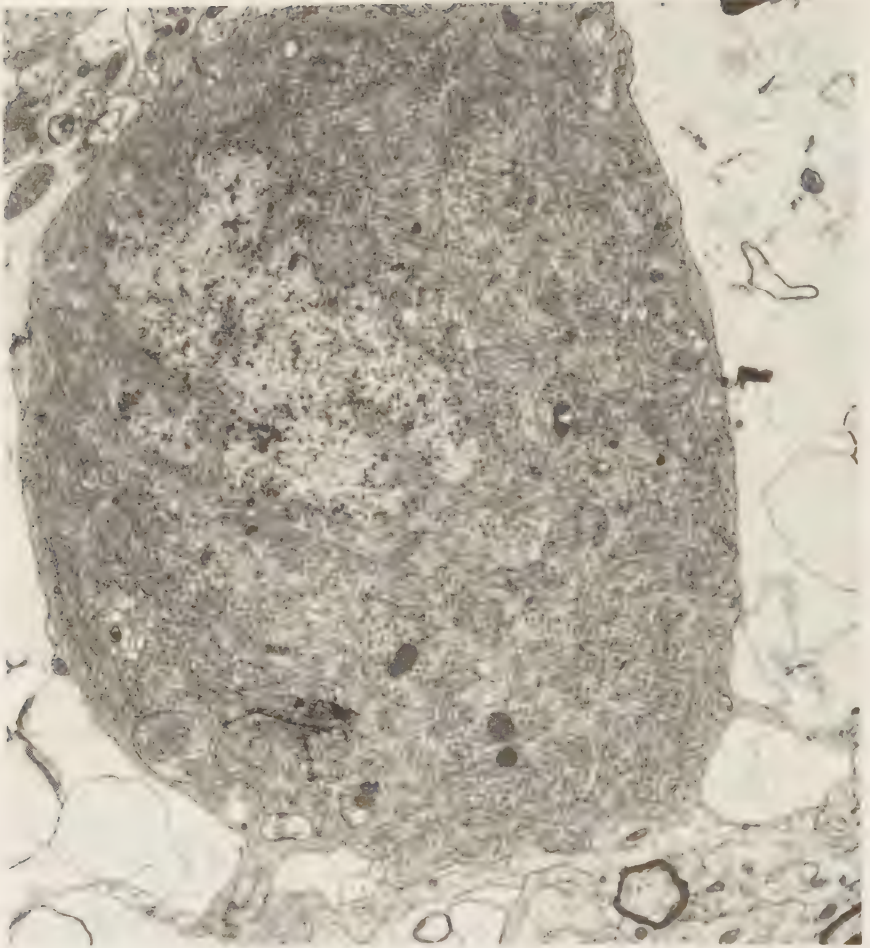


Fig. 7. This cell due to its localization and nuclear configuration is most likely a pyramidal basket neuron. The major alterations are changes in the perikaryon and increased electron density of the cytoplasm. Magn. 3300X.

Table 2. Proportions of normal and abnormal (type 1 a, incip., 1 b) neurons in stratum granulosum of the fascia dentata (FD), CA₁, CA₃ and subiculum (subic.) or rats with bicuculline-induced status epilepticus for 1 or 2 h. One group of rats was allowed to recover for 2 h after a seizure period of 1 h. Consecutively found neurons were classified according to criteria given in Material and Methods. In FD only granular neurons were counted.
n = number of rats. Figures within brackets = ranges.

Area	Type of neurons	Status ep., 2 h without recovery % n=7	Status ep., 1 h without recovery % n=4	Status ep., 1 h with recovery 2 h % n=5
FD	normal	50.1 (30-56)	52.5 (48-56)	99.8 (99-100)
	1 a (incip.) 1 b	49.9 (44-70) 0	47.5 (44-52) 0	0.2 (0-1) 0
CA ₁	normal	7.4 (4-10)	8.3 (7-11)	94.4 (83-100)
	1 a (incip.) 1 b	91.9 (89-96) 0.7 (0-3)	88.9 (89-90) 2.9 (2-4)	5.0 (0-17) 0.6 (0-3)
CA ₃	normal	2.7 (2-4)	2.3 (1-3)	91.2 (80-100)
	1 a (incip.) 1 b	97.0 (95-98) 0.3 (0-1)	97.0 (94-99) 0.7 (0-3)	7.8 (0-20) 1.0 (0-5)
Subic.	normal	5.2 (5-7)	2.9 (2-4)	95.6 (83-100)
	1 a (incip.) 1 b	93.1 (92-95) 0.7 (0-2)	94.7 (93-98) 2.4 (2-4)	3.6 (0-17) 0.8 (0-4)

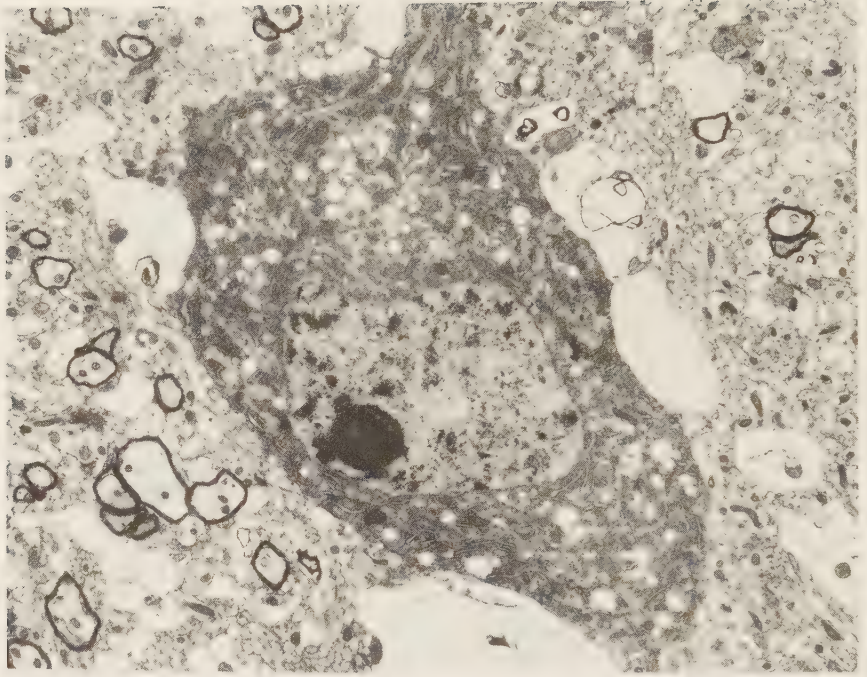


Fig. 8. Stratum polymorpha from the dentate gyrus of a rat with status epilepticus for 2 h. This pyramidal neuron is markedly changed with pronounced electron density of the cytoplasm and clumping of nuclear chromatin. Note the presence of cytoplasmic vacuoles and the presence of swollen astrocytic processes around the neuron. The vacuoles might partly be Golgi-derived, others may represent ballooned mitochondria. Magn. 3000X.

mixture of dendrites, axons, some of which are myelinated, and glial cell processes, apart from a few neuronal perikarya of quite varying configuration (Amaral 1978).

STATUS EPILEPTICUS. Rats subjected to status epilepticus for 1 and 2 h showed clear changes in the dentate gyrus (Fig. 3-8) but of varying intensity and character in the three different layers. At 2 h stratum moleculare displayed a slight, generalized astrocytic edema (Fig. 4), somewhat more pronounced in the central zone close to the granule cells. All dendrites were normal including their mitochondria, but a detailed study on their synapses has not yet been carried out.

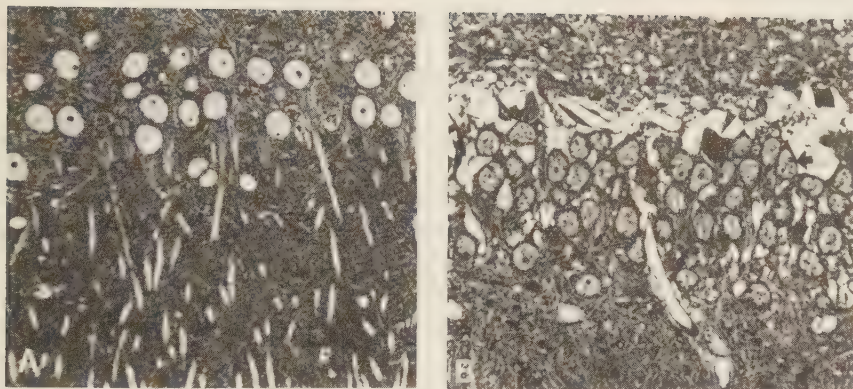


Fig. 9. LM appearance of the hippocampus CA₁, in a control (a) and a rat with status epilepticus for 2 h (b). Note the astrocytic edema and nerve cell changes. Most neuron had mild configurational changes (arrow) but some shrunken dark-staining neurons were also seen (arrowhead).

At 2 h stratum granulosum showed a marked astrocytic swelling with widely distended processes surrounding the blood vessels and the nerve cell bodies. The astrocytic nuclei appeared usually enlarged with evenly dispersed chromatin, i.e. changes of the same kind as seen in the fronto-parietal cortex (Söderfeldt et al. 1981). A large proportion of the granule cells showed slight changes characterized by increased electron density of the cytoplasm, irregular configuration of the soma and of the nuclear membrane with occasional marked infoldings. Their mitochondria were normal and only a few cells had slightly dilated rough endoplasmic reticulum. Very dark neurons and vacuolated granule cells of the kind previously described in the fronto-parietal cortex were only exceptionally encountered (<1%). However, in distinct contrast to the granule cells the few pyramidal basket neurons present in stratum granulosum showed pronounced changes either of the dark (1) or of the vacuolated type (2) with dilated portions or the rough endoplasmic reticulum (cf. Söderfeldt et al. 1981).

Stratum polymorphe contained pyramidal neurons with the same abnormalities, i.e. type 1 (Fig. 7) and 2. Virtually all the pyramidal neurons seen electron microscopically in this layer showed either type of change but their total number was few in relation to the entire

nerve cell population in the dentate gyrus. The astrocytic edema was very marked in stratum polymorphe.

Rats allowed to survive for up to 2 h after a seizure period of 1 h showed a remarkable recovery of the astrocytic edema and the nerve cell changes (Fig. 3b). For instance, at 2 h almost all the LM changes in the granule neurons had disappeared and the only remaining changes were the severe type 1 b changes in pyramidal basket neurons and the neurons bordering the CA 4 area.

HIPPOCAMPUS (cornu ammonis)

NORMAL. The main layers of the rat hippocampus (Figs. 1) have been described by Blackstad (1956) and by Raisman et al. (1965). The most superficial layer is stratum moleculare which contains the terminal dendritic plexus of the hippocampal pyramidal neurons and myelinated fibres from the enthorhinal cortex. Stratum radiatum contains the main shafts of the apical dendrites from the pyramidal cells which are closely packed in radial rows. The most conspicuous layer seen by LM is the stratum pyramidale, (Fig. 9a) which contains the perikarya of the pyramidal neurons and a few pyramidal basket cells adjacent to stratum oriens. In addition, astrocytes and blood vessels are present in this as well as in the other layers.

The pyramidal neurons have an extensive receptive field in the form of apical dendrites terminating in stratum moleculare and basal dendrites which extend into the stratum oriens (Lorente de N6 1934; Blackstad 1963; Raisman et al. 1965). Their axons form the hippocampal efferent and commissural systems passing to the deepest layer of the hippocampus, the alveus, which is dominated by the presence of numerous myelinated axons.

On the basis of cytoarchitectonic organization Lorente de N6 (1934) divided the cornu ammonis into four zones, referred to as fields CA 1, 2, 3 and 4. Field CA 1 contains two layers of fairly small pyramidal neurons, one superficial layer of one to two rows of packed cells and a deeper layer of irregularly scattered neurons (Westrum and Blackstad 1962; Raisman et al. 1965). CA 1 and CA 2 contain in addition a narrow zone between the molecular layer and the stratum radiatum termed stratum lacunosum which harbours the so-called Schaffer collaterals from CA 3 and CA 4 neurons.

Field CA 3 contains the largest pyramidal neurons. The proximal part of their apical dendrites has thick thorn-like excrescences (Blackstad 1963) which are in contact with the mossy fibre endings from the dentate neurons. Therefore, in this field there is a narrow zone superficial to the stratum pyramidale termed stratum lucidum.

STATUS EPILEPTICUS. By LM stratum pyramidale from CA 1 to CA 3 as well as the pyramidal cell area of CA 4 showed a very marked status spongiosus (Fig. 9b). This change was seen in all the rats with status epilepticus for 1 or 2 h. It was somewhat more marked in the zone adjacent to stratum oriens, and in rats with status epilepticus for 2h it frequently occupied this layer as well. EM confirmed the presence of edema and showed that the LM appearance of status spongiosus corresponded to swollen astrocytic processes (Fig. 10). Signs of astrocytic swelling were also found in the other layers, but was much less pronounced. Commonly, in all the layers astrocytic nuclei were similarly enlarged with evenly dispersed chromatin as in fascia dentata (Fig. 5).

Nerve cell changes (Fig. 9b) were present throughout CA 1 to CA 4 but in general they appeared less severe than those in the fronto-parietal cortex. The vast majority of the pyramidal neurons had only mild to moderate configurational changes and they stained with toluidine blue somewhat darker than normal neurons (incipient type 1 a change. Some shrunken, dark staining neurons (type 1 b) were seen in all epileptic animals but they were equally infrequent in areas CA 1 - CA 3 (<1%), only in CA 4 were they more frequent. All type 1 injured neurons (Figs. 10 and 11) had basically the same ultrastructural characteristics as presented before (Söderfeldt et al. 1981), e.g. in type 1 a change (Fig. 10) the perikarya had more or less normal organelles, irregular outlines of the plasma membrane due to swollen astrocytic processes and occasional infoldings of the nuclear membrane. A few cells had dilated rough endoplasmic reticulum as in the type 2 injury and exceptionally we found neurons with abnormal, distended mitochondria.

With few exceptions recovery animals showed by LM at 2 h survival after the end of a 1 h seizure period only minimal changes (Fig. 9c). The incipient type 1 a neuronal changes as well as the

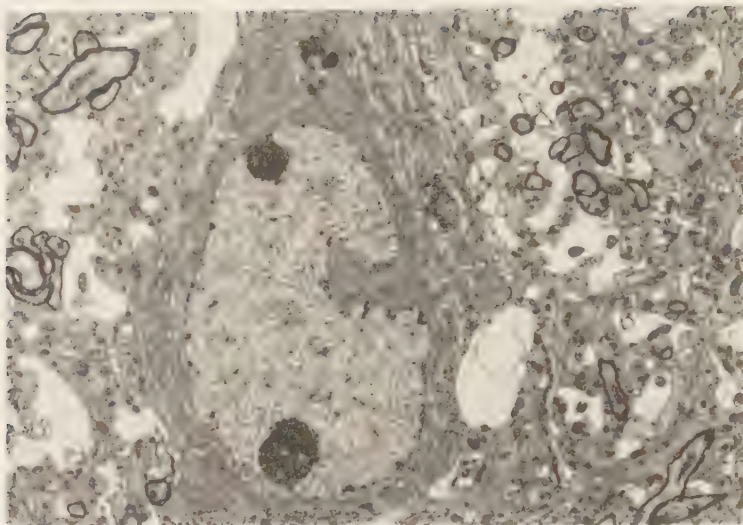


Fig. 10. Pyramidal nerve cell from CA₃ of a rat with status epilepticus for 2 h. This neuron with incipient type 1a change shows only minor configurational changes and slight increased electron density of the cytoplasm. Magn. 2300X.

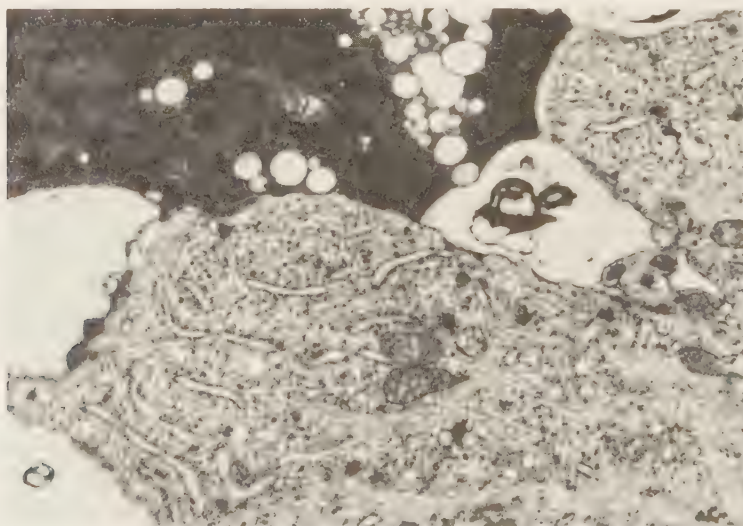


Fig. 11. Parts from two pyramidal neurons (CA 1) obtained from the same rat as in Fig. 10. The upper dark stained neuron (1b) is severely changed (type 1b) whereas the lower nerve cell has a normal appearance apart from a slight dilatation of rough endoplasmic reticulum and slight mitochondrial changes. Magn. 7900X.

edema, had almost entirely vanished. In one animal (Table 2) nerve cell changes persisted in a small rounded edematous area (zone with vasogenic edema due to breakdown of the blood-brain barrier?). A couple of nerve cells with severe alterations (type 1 b) remained by they were few in number as in the rats without recovery.

SUBICULUM

NORMAL. The subiculum is the cortical area which is connected by the prosubiculum to the hippocampus. It is characterized by a single layer of large, loosely arranged pyramidal neurons (Blackstad 1956). The border towards the CA 1 region is rather well defined since the pyramidal cells of the hippocampus become loosely apposed in the CA 1 area. As in CA 1 region there is also a covering molecular layer.

STATUS EPILEPTICUS. Animals with status epilepticus for 1-2 h showed neuronal changes of similar extent and quality as in the adjacent CA 1 region. In recovery animals the incipient type 1 a change was no longer present but in a few rats (1 h of status epilepticus and 2 h recovery) type 1 b nerve cell changes remained.

DISCUSSION

It seems justified to confine the discussion to three major aspects of the present results, (1) their relations to previous data on seizure-induced changes in experimental epilepsy, (2) alterations in hippocampal versus cerebral cortical cells and (3) changes affecting the various areas of the hippocampal formation.

1. Hippocampal changes in experimental epilepsy

As stated in the introduction, damage to hippocampal neurons is a common finding in patients with epilepsy. Some experimental studies also attest the vulnerability of these cells to sustained seizure activity. For example, Purpura and Gonzalez-Monteagudo (1960) found that seizures of 2-6 h duration induced by the anti-metabolite methoxypyridoxine caused extensive cell damage in the CA 3 - CA 4 regions. Furthermore, in a recent article marked neuronal damage in the hippocampal formation has been described following limbic seizures induced by i.v. injected kainic acid (Lothman and Collins 1981). Other results suggest that such hippocampal cell damage is not caused by the enhanced neuronal activity per se but relates to the type of con-

vulsant used. Thus, in the study of Purpura and Gonzales-Montegoudo (1960) damage was seen with seizures induced by methoxypyridoxine but not by strychnine or metrazol.

The present study has shown that hippocampal changes develop when seizures of 1-2 h duration are induced by the GABA-receptor blocker agent bicuculline. The results are thus in line with previous LM observations of baboons (Meldrum and Brierley 1973; Meldrum et al. 1973) and rats (Blennow et al. 1978) as well as with a recent brief LM and EM report on hippocampal changes in the rat (Griffiths et al. 1982). However, in the cat model used by Brown et al. (1980) no hippocampal damage was detected by LM or EM observations in spite of the fact that seizures were induced for several hours. Probably, the difference in results is more apparent than real. Thus, Brown et al. (1980) allowed recovery periods of 1 h or more before the tissue was fixed by perfusion. Furthermore, in the study of Meldrum et al. (1974) only one animal was allowed a significant recovery period and in this one (with 7 h of seizure activity) hippocampal damage was not observed. The present result also demonstrate that only slight alterations remain when seizures are arrested for 2 h prior to fixation by perfusion. Therefore, it seems probable that seizures induced by bicuculline do not readily cause extensive hippocampal damage.

2. Hippocampal versus cerebral cortical damage

It has been clearly shown that metabolic changes in the hippocampus during bicuculline-induced seizures are at least as pronounced as in the cerebral cortex (Folbergrová et al. 1981; Siesjö et al. 1982) and, on a relative basis, limbic structures show the most marked changes in local metabolic rate (Ingvar and Siesjö 1983). In view of such results one would expect similar structural alterations in the hippocampus as in the cerebral cortex, if these alterations directly reflect the metabolic perturbation.

The comparison between structural alterations in the hippocampus and fronto-parietal cortex is facilitated by the fact that the tissues were obtained from the same animals. Obviously, changes recorded after 1 or 2 h of seizure activity were - with the exception of the astrocytic edema - much less pronounced in the hippocampus than in the cerebral cortex. Thus, although type 1 and type 2 nerve cell changes were also recognized in the hippocampal formation they were much less common, and in the dentate gyrus and stratum pyramidale of

the hippocampus the swollen, vacuolated type 2 neurons were extremely rare. Instead, the most common abnormality was so-called incipient type 1 a change, i.e. a neuron with irregular outlines and increased staining of the cytoplasm.

The very dark and shrunken neurons of type 1 b were indeed rare but when present, had basically the same LM and EM characteristics as described for fronto-parietal cortex (Söderfeldt et al. 1981). The EM characteristics of these cells were pronounced electron density and the presence of multiple groups of cytoplasmic vacuoles. As discussed before, these vacuoles may represent dilated Golgi cisternae and, at least in part, swollen mitochondria (Söderfeldt et al. 1981). Similar condensed, microvacuolated pyramidal neurons were apparently noted by Griffiths et al. (1982) in their preliminary LM and EM report.

A recent study from our laboratories has shown that when seizures are arrested by i.v. diazepam and the brains perfused after a recovery period for 1 or 2 h the vast majority of the type 1 nerve cell changes and all type 2 changes disappear in cerebral cortex (Söderfeldt et al., in preparation).. It is therefore tempting to conclude that these changes mainly reflect reversible alterations of configuration and staining characteristics, induced by the seizure activity and that they do not indicate irreversible cell damage. If we let aside the unknown factors, leading to more pronounced changes in the cerebral cortex than in the hippocampus, we submit that the relevant comparison should be made between recovery groups. We then find that a comparable amount of nerve cells (about 1 per cent) show signs of permanent damage both in cerebral cortex (Söderfeldt et al. 1981) and hippocampus (present data). However, we wish to recall that such a comparison is only tentative since definitive conclusions will require seizure models that allow much more extensive recovery periods. For example, at this moment we do not know if the return of cell shape and staining qualities signifies complete recovery in the sense that the neurons are comparable to their response to further insults, nor do we know whether they will show degenerative changes days or weeks after the initial insult.

3. Selective vulnerability within the hippocampal formation

It is a well-known fact that neurons in the various regions of the hippocampal formation vary with respect to their reaction towards

pathological influences, i.e. they display a form of selective vulnerability. In this study we found that the most pronounced astrocytic swelling occurred in areas where the neuronal perikarya are located i.e. the stratum granulosum of the dentate gyrus and the stratum pyramidale of the hippocampus. The results also showed that although astrocytic swelling and the incipient type 1 a nerve cell changes affected neuronal populations of all the CA areas, the most severe and presumably at least in part irreversibly damaged type 1 b neurons were most constantly present in CA 4 rather than in the Sommer sector (CA 1).

A previously unrecognized selective vulnerability seemed to affect the dentate gyrus. Whereas the granule neurons usually were only slightly changed the pyramidal basket neurons, present in the deeper part of the stratum granulosum, showed such severe alterations that they presumably are not compatible with survival of the cells. These neurons are most probably GABA-ergic and thus provide inhibition to other neurons (Ribak and Andersson 1980). It would be of great interest to find out whether this cell type shows a similar selective vulnerability in other models of status epilepticus, or in other pathological conditions, since a selective damage to these inhibitory neurons might well have functional implications in the period after a transient pathological influence. For example, one could hypothesize that a selective destruction of such inhibitory neurons facilitates further epileptic discharges from the hippocampus.

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Metabolic, circulatory, and structural alterations in
the rat brain induced by sustained pentylenetetrazol seizures.

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ABSTRACT

Previous studies have demonstrated that bicuculline-induced seizures of 1-2 h duration lead to structural alterations in the rat brain, and they have provided extensive information on the accompanying cerebral metabolic and circulatory changes. Such alterations, which affected both neurons and glial cells, were observed even though cerebral oxygenation seemed adequate and at least some of them persisted after arrest of seizure activity, suggesting that irreversible neuronal damage had been incurred in the neocortex and the hippocampal formation. In the present study, we explored whether pentylenetetrazol, ("Metrazol"), a convulsant which has been reported to interfere with GABA inhibition by mechanisms other than that of bicuculline, leads to similar structural alterations and to similar cerebral metabolic and circulatory changes. The drug was given to paralyzed and artificially ventilated rats in a dose of $100 \text{ mg} \cdot \text{kg}^{-1}$ i.v., and seizures were allowed to continue for 1-120 min to allow assessment of changes in EEG pattern, in labile phosphates, glycolytic metabolites, and cyclic nucleotides (1, 60, and 120 min), in free fatty acids (1,5,60,120 min) and structural changes (120 min).

The onset of seizures was accompanied by a small perturbation of cerebral cortical energy state, but sustained changes were confined to decreases in phosphocreatine, glycogen and glucose, and increases in lactate, pyruvate and cyclic nucleotides. A sustained increase in free fatty acid concentration was observed, the largest change occurring in arachidonic acid concentration. In the cerebellum, metabolic perturbation was clearly less pronounced but cyclic nucleotide concentrations rose substantially. Local cerebral blood flow increased in all but two structures (frontal cortex and caudoputamen) but pronounced interstructural changes occurred, and no decrease in CBF was observed in the period 60 to 120 min. Nerve cell changes and astrocytic swelling, were observed in the cerebral cortex. There was marked status spongiosus due to edema which was mainly astrocytic and most prominent in cortical layer 3. Nerve cell changes were of two basic types. The type 1 injured neurons, condensed and triangular in shape, were mainly confined to the edematous areas. Many of them had cytoplasmic vacuoles which in electron microscopy proved to be mainly dilated Golgi cisternae or mitochondria. As compared with bicuculline-induced epilepsy such abnormal mitochondria appeared to be more frequent. The type 2 neurons had slit-formed intracytoplasmic and perinuclear vacuoles resulting from dilatation of the endoplasmic reticulum cisternae and the nuclear envelope. Also in the hippocampus a marked status spongiosus and nerve cell changes were seen, but the cerebellum looked light microscopically normal.

We conclude that, in the rat, sustained seizure activity induced by metrazol is accompanied by similar alterations in EEG activity, in cerebral metabolism and circulation, and in cell structure as those elicited by bicuculline.

INTRODUCTION

Although it has been established that sustained and/or recurrent seizures in patients may give rise to neuronal cell damage in the brain (e.g. Scholz 1959, Norman 1964, Corsellis and Meldrum 1976), it has remained a matter of speculation whether this damage is caused by the seizure activity per se or if complicating systemic or cerebral events are responsible or, at least, contributory (see Meldrum et al. 1973, Blennow et al. 1978, Söderfeldt et al. 1982a). In the clinical setting, seizures are often accompanied by arterial hypoxia and/or hypotension, by hypoglycemia, and by hyperthermia. Such complications may curtail oxygen and glucose supply to the intensely discharging neurons. In addition, hypotension may, especially if combined with swelling of cellular elements, prevent a purposeful increase in CBF, or even jeopardize microcirculation at critical tissue loci. It is natural, therefore, that experimental attempts have been made to induce longlasting seizures under conditions which allow control of systemic variables.

Light microscopic (LM) and/or electron microscopic (EM) analysis of structural alterations caused by epileptic brain discharge has been carried out following induction of seizures with electroshock or chemical convulsants. Although one group of workers reported that 7-10 electroshock seizures gave rise to altered configuration of neuronal elements (Mölbart et al. 1967) subsequent workers, utilizing more adequate tissue fixation techniques, have failed to find that single or repeated electroshock seizures alters tissue structure (Brennan et al. 1972, Mouritzen-Dam et al. 1980).

Experiments with chemical convulsants offer the advantage that a continuous seizure discharge can be sustained for long periods. The evidence that sustained seizures give rise to cell damage, seemingly unrelated to systemic complications, derives from experiments with methoxyypyridoxine (Purpura and Gonzales-Montegudo 1960), allylglycine (Meldrum et al. 1974, Griffiths et al. 1982), bicuculline (Meldrum et al. 1973, Blennow et al. 1978, Söderfeldt et al. 1981, Attilo et al. 1983) and kainic acid (Lothman and Collins 1981, see also Ben-Ari et al. 1981). However, extensive correlations to systemic events and to cerebral metabolic-circulatory changes have only been made in seizures

induced by bicuculline.

Although contradictory results have been reported, and although it remains uncertain to what extent structural alterations persist following arrest or seizure discharge (see Discussion), experiments on ventilated and physiologically controlled baboons and rats indicate that bicuculline-induced seizures of at least 1 h duration give rise to neuronal cell damage in the cerebral cortex, the hippocampus and the striatum, but not in the cerebellum (Meldrum et al. 1974, Blennow et al. 1978, Söderfeldt et al. 1981, Griffith et al. 1982). In the rat, extensive information on bicuculline-induced changes exist on overall as well as local cerebral blood flow and metabolic rate (Meldrum and Nilsson 1976, Borgström et al 1976, Horton et al. 1980, Ingvar and Siesjö 1982), on energy, carbohydrate, amino acid and protein metabolism (Chapman et al. 1977, Blennow et al. 1979, Folbergrova et al. 1980, Kiessling and Kleihues 1981), and on free fatty acids (Siesjö et al. 1982). It is of interest that, in the bicuculline model, metabolic changes are more pronounced in the neocortex and hippocampus than in the cerebellum (Folbergrova et al. 1980), also that the initially marked hyperemia is clearly attenuated in "vulnerable" structures in spite of maintained seizure discharge and metabolic rate (Ingvar and Siesjö 1982).

The question arises whether changes observed during bicuculline-induced seizures are specific for this particular drug or representative of also other types of "generalized" seizures. We decided, therefore, to explore corresponding changes induced by the commonly employed convulsant pentylenetetrazol. Although this drug, like bicuculline, is classified as a GABA antagonist, it does not seem to act as a GABA receptor blocker (Marangor et al. 1979, Simmons 1980). Information exists on pentylenetetrazol-induced changes in labile cerebral tissue metabolites (Tews et al. 1965, Duffy et al. 1975), in free fatty acids (Bazan 1971, Marion and Wolfe 1978), and in the cerebral metabolic rate/blood flow couple (Plum et al. 1968). However, since these studies give no information on sustained seizures, since FFA concentrations were measured only in animals with unassisted ventilation, and since no data exist on local blood flow rates, we assessed labile metabolites, including free fatty acids, in the cerebral cortex and the cerebellum after seizure durations of 1 - 120 min, measured local CBF in 25 different structures, and analysed light

and electromicroscopic changes in the cerebral cortex after 120 min of continuous seizures.

MATERIALS AND METHODS

1. Animals and experimental groups

The experiments were all performed on male Wistar rats weighing between 345 and 405 g. The animals had free access to food (Sanbolagen, Malmö, Sweden) and water until the time of operation.

There were three main series of animals. In the first series consisting of four subgroups (control, 1 min, 60 min and 120 min of metrazol-induced seizures) cerebral cortical and cerebellar energy metabolites and cyclic nucleotides were measured. On the hemisphere not used for these analyses the cortical content of free fatty acids were analyzed. An additional group of animals subjected to 5 min of seizure activity was also included in the analyses of free fatty acids.

The second series also consisted of four subgroups. Here, local cerebral blood flow was measured in the control state as well as after 20, 60 and 120 min of ongoing seizure activity.

The third series comprised 6 animals that were perfusion fixed after 120 min of seizure activity. Another 4 animals without seizures, maintained under anaesthesia for a similar period of time, served as controls.

2. Operative and sampling techniques

a. Procedures for measuring tissue metabolites

The animals were anesthetized in a jar with a mixture of 3% halothane and 75% N₂O in oxygen. When unresponsive, they were tracheostomized, paralyzed with tubocurarine (1.5mg·kg⁻¹) and ventilated on a Starling type respirator. The ventilator was set to yield an arterial PCO₂ of between 35 and 40 mm Hg prior to induction of seizures. The halothane concentration was then reduced to 0.7% until the operation was completed. The femoral vessels were cannulated for blood pressure recording, blood sampling, and infusion of drugs. The bone of the skull was exposed through a skin incision. In all experiments two gold plated EEG screws were inserted in the bone in the parietal region and the EEG was recorded continuously (Siemens Elema,

Stockholm). A plastic funnel was placed in a skin incision over the exposed skull bone, making it possible to later freeze the brain in situ by pouring liquid nitrogen into the funnel. Blood gases were evaluated on samples of 150 μ l (Eschweiler and Co., Kiel, and Radiometer, Copenhagen). Rectal temperature was maintained close to 37° C with a heating lamp. The animals were heparinized and allowed to recover for at least 30 min before induction of seizures. Pentyl-enetetrazol was then injected i.v. (100 mg.kg⁻¹) following a lowering of the blood pressure of the animal to approximately 100 mm Hg by slow withdrawal of 3-5 ml 1-blood. For details of procedures, see Ingvar and Siesjö (1982).

b. Procedures for measuring CBF

The method used for measuring local cerebral blood flow was the one described by Sakurada et al (1978), with procedures identical to those presented elsewhere (see Ingvar and Siesjö 1982). In short, ¹⁴C-iodoantipyrine was infused over a time period during which the arterial concentration of the radioactive tracer was monitored by 10-12 timed arterial blood samples. The animal was decapitated immediately after the last blood sample. The brain was rapidly removed, frozen, and later sectioned in a freeze microtome. The slices thus obtained were dried and allowed to expose an X-ray film for one week, together with a set of calibrated ¹⁴C standards. The staining of the film was then manually evaluated with a densitometer. The concentration of the tracer in the blood samples was calculated using β scintillation using internal standard for evaluation of efficiency. Calculation of the blood flow was done according to Sakurada et al 1978.

3. Methods and analytical techniques

a. Labile phosphates, glycolytic metabolites and cyclic nucleotides

Samples of cerebral cortex and hippocampus were extracted with HCl-methanol at -22°C, and subsequently with perchloric acid at 0°C. Tissue contents of glycogen, glucose, pyruvate, lactate, phosphocreatine (PCr), ATP, ADP, and AMP were measured with enzymatic, fluorometric techniques (for information on analytical conditions, see Folbergrova et al 1980). A protein binding technique was used to measure cAMP. For measuring cGMP we used a radioimmunoassay technique. For this metabolite, an acidic extract (pH 6.2) was prepared (Folbergrova et al 1980).

b. Free fatty acids

For assaying free fatty acids we used conventional techniques, as previously described (Rehncrona et al 1980). In summary, the frozen tissue samples (about 100 mg) were homogenized in at least 26 volumes of chloroform-methanol-water (1:1:0.2, v/v) with BHT (2,6-ditert-butyl-p-cresol) added as an antioxidant. After further processing of the samples, the FFA fraction was separated on silica gel 60 with light petroleum:diethyl ether:acetic acid (55:45:2 v/v) as solvents. The FFA band was visualized in UV-light and scraped off the plates. The FFA were then esterified, and the methylesters separated by GLC and quantified with an automatic integrator. Heinecosanoic acid was added as an internal standard.

c. Calculations

Energy charge of the adenylate pool (EC) was calculated according to Atkinsson (1968). The local cerebral blood flow was calculated according to Landau et al (1955). Statistical differences were evaluated using Student's t-test.

4. Histopathological methods

After 2 h of status epilepticus the brains were fixed by vascular perfusion through a metal cannula placed in the ascending aorta after a brief rinse with saline. A detailed description of the fixation procedure and the glutaraldehyde fixative used is presented in a previous paper (Söderfeldt et al. 1981). Briefly, a 3 % fixative of glutaraldehyde in 0.1 mol.l^{-1} phosphate buffer, pH 7.4, 37 °C, was used. Perfusion pressure was 135 mmHg. After perfusion the brain was left in situ for at least 45 min; it was then removed and stored in fixative until processed for microscopy. Efficiency of fixation was evaluated by absence of blood in vessels. One hemisphere of the brain and the cerebellum were coronally sectioned and embedded in plastic (EFL-67, Serva Feinbiochemica, Heidelberg, FRG). From the other hemisphere samples were taken for EM and for embedding in paraffin. The EFL-67 embedded sections were stained with toluidine-blue and the paraffin sections with hematoxylin-eosin and cresyl violet.

The cortical tissue blocks for EM were sectioned coronally into 230 μm slices with a Sorvall TC-2 tissue sectioner and processed for EM with embedding in epon. Semithin sections were then stained with toluidine blue for high resolution LM, and used for the selection of

areas for thin sections. These were double stained with uranyl acetate and lead citrate and examined in a JEM 100 C electron microscope. The number of injured neurons in the cortex was counted with help of an eye piece graticule at 40x magnification in 250 μ m wide strips of cerebral cortex.

RESULTS

As described above, there were three series of animals used for measurements of tissue metabolites (1, 5, 60, and 120 min of seizure activity), local blood flow (20, 60, and 120 min), and histopathology (120 min). In all animals included in the series, the seizures were sustained for the periods chosen. Fig. 1 illustrates typical changes in EEG pattern following the i.v. injection of pentylenetetrazol.

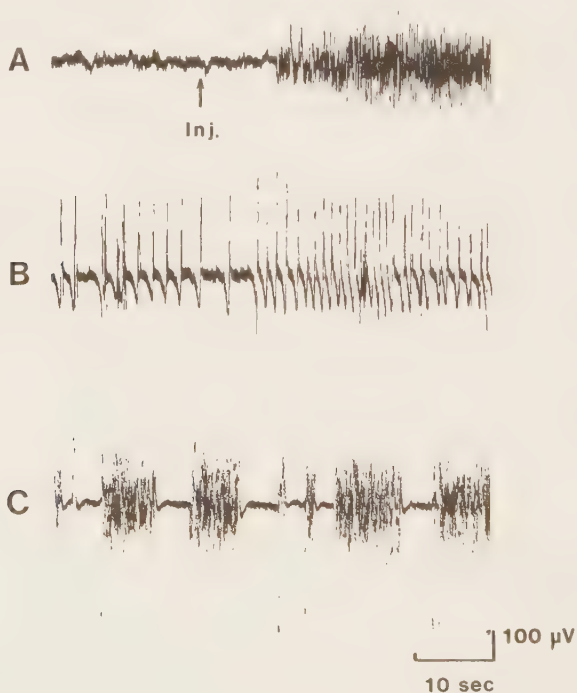


Fig.1. Changes in EEG activity following intravenous administration of pentylenetetrazol in paralysed and mechanically ventilated rat. A. At injection. B. After 1'. C. After 60'.

Isolated spikes appeared within the first 10 sec, followed by an intense seizure discharge. After 2 to 10 min, the pattern changed to one of bursts of spikes, separated by periods of EEG suppression. This pattern was then sustained until termination of the experiments. The EEG changes were thus similar to those recorded following induction of seizures with bicuculline (see Chapman et al. 1977).

Parameters recorded in the tissue metabolite series are listed in table 1. The injection of pentylenetetrazol, and initiation of seizure discharge, led to a transient increase in blood pressure. However, due to the initial withdrawal of blood, peak pressures did not exceed 160 mm Hg. After about 5 to 10 min, the pressure fell spontaneously. In order to maintain blood pressure above 110 mmHg the previously shed blood plus blood from donor rats were infused at later stages. Since arterial P_{O_2} values were upheld, and body temperature could be maintained at around $37^{\circ}C$, it was thus possible to avoid the common complications of status epilepticus (hypotension, hypoxia, and hyperthermia). However, like bicuculline-induced seizures (see Chapman et al. 1977) those provoked by pentylenetetrazol were accompanied by severe plasma acidosis.

	Control	1'	5'*	60'	120'
MABP	148 $\pm$ 5	126 $\pm$ 3	135 $\pm$ 2	117 $\pm$ 2	114 $\pm$ 4
Peak BP	---	152 $\pm$ 5	171 $\pm$ 4	155 $\pm$ 8	151 $\pm$ 6
pO_2	106 $\pm$ 4	107 $\pm$ 2	102 $\pm$ 3	98 $\pm$ 3	109 $\pm$ 5
pCO_2	35.4 $\pm$ 0.4	27.4 $\pm$ 1.4	34.8 $\pm$ 2.6	34.7 $\pm$ 0.8	36.6 $\pm$ 1.5
pH	7.39 $\pm$ 0.02	7.45 $\pm$ 0.02	7.30 $\pm$ 0.04	6.93 $\pm$ 0.03	7.06 $\pm$ 0.04
Temp	36.8 $\pm$ 0.2	36.7 $\pm$ 0.1	37.0 $\pm$ 0.2	36.7 $\pm$ 0.2	36.9 $\pm$ 0.2

*in this group only FFA was analyzed.

Table 1. Physiological variables for animals in the tissue metabolite series

In animals used for I-CBF measurements, and for histopathological analyses, EEG changes and physiological parameters were similar to those shown in table 1 (data not given).

1. Labile phosphates, glycolytic metabolites, and cyclic nucleotides

Table 2 gives values measured in control animals, and in those with seizure periods of 1, 60, and 120 min for both the cerebral cortex and the cerebellum.

In the cerebral cortex, the onset of seizure discharge (1 min) was associated with significant reductions in PCr and ATP concentrations, with an associated, small reduction in adenylate energy charge. Glucose concentration was markedly lowered, and glycogen concentration reduced. Both lactate and pyruvate concentrations were increased but not proportionally; hence an increase in the lactate/pyruvate ratio occurred. Cyclic AMP concentration was raised 3- to 4-fold, and CGMP concentration doubled.

With prolongation of the seizure discharge for 60 and 120 min there was a persisting reduction in PCr concentration but not in ATP concentrations. Although the mean energy charge value was reduced, the values did not significantly differ from control. Both the lactate concentration and the lactate/pyruvate ratio remained elevated, as did the CGMP concentration. cAMP concentrations were clearly reduced, as compared to the 1 min value, but nevertheless remained increased above control.

In the 60 min and 120 min groups one and two animals, respectively, had tissue glucose concentrations below 0.5 mmol.g^{-1} . In these three animals, lactate concentrations were 4.1, 4.1, and 1.7 mmol.g^{-1} , respectively, indicating that such low glucose concentrations are limiting for lactic acid production (see Folbergrova et al. 1980). Results obtained in the remaining animals (tissue glucose concentrations $> 0.9 \text{ mmol.g}^{-1}$) showed that, in the absence of substrate limitation, tissue lactate concentrations slightly exceeded 10 mmol.g^{-1} after 1 and 2 h of seizure activity.

Metabolite changes in the cerebellum were different. Thus, although some decrease in PCr concentration was noted after 1 min, concentrations of labile organic phosphates did not differ from control after 60 and 120 min. Furthermore, although some initial decrease in glucose concentration was observed, the 60 and 120 min values were in the control range; it should also be noted that gly-

	Control	1 min	60 min	120 min
Phosphocreatine	4.61 $\pm$ 0.09	3.1 $\pm$ 0.13***	2.91 $\pm$ 0.14***	3.01 $\pm$ 0.17***
ATP	2.56 $\pm$ 0.05	2.37 $\pm$ 0.04*	2.43 $\pm$ 0.05	2.49 $\pm$ 0.06
ADP	0.31 $\pm$ 0.01	0.32 $\pm$ 0.07	0.32 $\pm$ 0.01	0.36 $\pm$ 0.03
AMP	0.05 $\pm$ 0.01	0.05 $\pm$ 0.00	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01
E.C.	0.93 $\pm$ 0.00	0.92 $\pm$ 0.00*	0.92 $\pm$ 0.00	0.92 $\pm$ 0.01
Glucose	3.69 $\pm$ 0.37	0.83 $\pm$ 0.11***	2.48 $\pm$ 0.56	1.15 $\pm$ 0.27***
Glycogen	2.51 $\pm$ 0.26	1.61 $\pm$ 0.21*	0.87 $\pm$ 0.15***	1.11 $\pm$ 0.25**
Lactate	1.89 $\pm$ 0.24	6.14 $\pm$ 0.22***	9.92 $\pm$ 1.64***	8.16 $\pm$ 1.84**
Pyruvate	0.11 $\pm$ 0.01	0.15 $\pm$ 0.01*	0.17 $\pm$ 0.01**	0.16 $\pm$ 0.02*
Lac/pyr	18.10 $\pm$ 2.70	43.20 $\pm$ 4.30***	59.50 $\pm$ 9.60**	47.30 $\pm$ 7.70**
cAMP	1.42 $\pm$ 0.06	5.83 $\pm$ 0.42***	1.72 $\pm$ 0.07**	1.77 $\pm$ 0.11*
cGMP	0.066 $\pm$ 0.014	0.118 $\pm$ 0.017*	0.214 $\pm$ 0.032**	0.181 $\pm$ 0.048*
Phosphocreatine	7.13 $\pm$ 0.15	6.37 $\pm$ 0.22*	6.92 $\pm$ 0.19	6.74 $\pm$ 0.17
ATP	2.47 $\pm$ 0.04	2.40 $\pm$ 0.05	2.53 $\pm$ 0.03	2.50 $\pm$ 0.05
ADP	0.23 $\pm$ 0.01	0.25 $\pm$ 0.01	0.25 $\pm$ 0.01	0.25 $\pm$ 0.01
AMP	0.04 $\pm$ 0.00	0.04 $\pm$ 0.00	0.04 $\pm$ 0.00	0.04 $\pm$ 0.00
E.C.	0.94 $\pm$ 0.00	0.94 $\pm$ 0.00	0.94 $\pm$ 0.00	0.94 $\pm$ 0.00
Glucose	4.64 $\pm$ 0.51	1.77 $\pm$ 0.18***	4.70 $\pm$ 0.89	3.04 $\pm$ 0.79
Glycogen	2.87 $\pm$ 0.29	1.76 $\pm$ 0.21*	1.31 $\pm$ 0.25**	1.86 $\pm$ 0.26*
Lactate	1.40 $\pm$ 0.10	4.93 $\pm$ 0.25***	3.54 $\pm$ 0.09***	2.64 $\pm$ 0.25**
Pyruvate	0.08 $\pm$ 0.01	0.16 $\pm$ 0.01***	0.11 $\pm$ 0.01**	0.11 $\pm$ 0.01*
Lac/pyr	18.10 $\pm$ 1.4	31.40 $\pm$ 3.00**	32.00 $\pm$ 2.10***	23.60 $\pm$ 1.20*
cAMP	1.05 $\pm$ 0.12	9.05 $\pm$ 1.48***	0.91 $\pm$ 0.06	1.13 $\pm$ 0.14
cGMP	0.62 $\pm$ 0.06	9.00 $\pm$ 0.58***	3.72 $\pm$ 0.57***	4.18 $\pm$ 0.85**

Values are given as means $\pm$ S.E. in μ mol/g except cyclic nucleotides which are given in μ mol/kg.

Table 2 Energy metabolites in cerebral cortex (upper) and cerebellum (lower) after 1, 60 and 120 min of status epilepticus.

cogen concentrations were only moderately reduced. Finally, less lactate accumulated in the cerebellum than in the cerebral cortex, with a less marked increase in the lactate/pyruvate ratios. Changes in cyclic nucleotides were remarkable. Thus, cAMP rose 8-fold after 1 min, and then returned to normal concentrations, while CGMP rose excessively after 1 min, and remained grossly elevated for the remainder of the 120 min seizure period.

2. Free fatty acids

As shown in Fig. 2, total FFA concentrations of cerebral cortical tissue rose to 150 % of control after 1 min, and to 200 % of control after 5 min. The values then subsequently fell, but significant elevations (to about 125 % of control) persisted at 60 and 120 min. Fig. 2 also shows that although changes in the concentrations of 16:0 and 18:0 contributed to the FFA accumulation, the largest percentage changes occurred in the concentrations of 18:0 and, especially, 20:4. The results demonstrate that, following its massive accumulation at 1 and 5 min, the concentration of arachidonic acid subsequently fell.

3. Local CBF

Local CBF values for 25 different brain structures are given in Table 3. In general, the seizures were accompanied by pronounced increases in local flow rates. However, relatively marked interstructural differences occurred. In two structures (frontal cortex and caudoputamen) significant hyperemia was observed only after 60 min, and in another three (sengorimotor, parietal, and auditory cortex) values during seizures were close to, but not consistently above 200 % of control. In most of the remaining structures, CBF values exceeded 250 % of control and, in seven, flow rates were consistently above 300 % of control (globus pallidus, nucleus ruber, thalamus, lateral thalamus, superior collicilus, cingulate cortex, and amygdyla). Two findings should be emphasized. First, although the metabolic perturbation was more pronounced in the parietal cortex than in the cerebellum, flow rates were, on a percentage basis, higher in the cerebellum. Second, prolongation of the seizure period from 60 to 120 min did not significantly reduce CBF in any of the structures examined (see Discussion).

4. Histopathology

Our four controls appeared both in LM and EM such as generally considered normal, including the Purkinje cell layer, (Peters et al

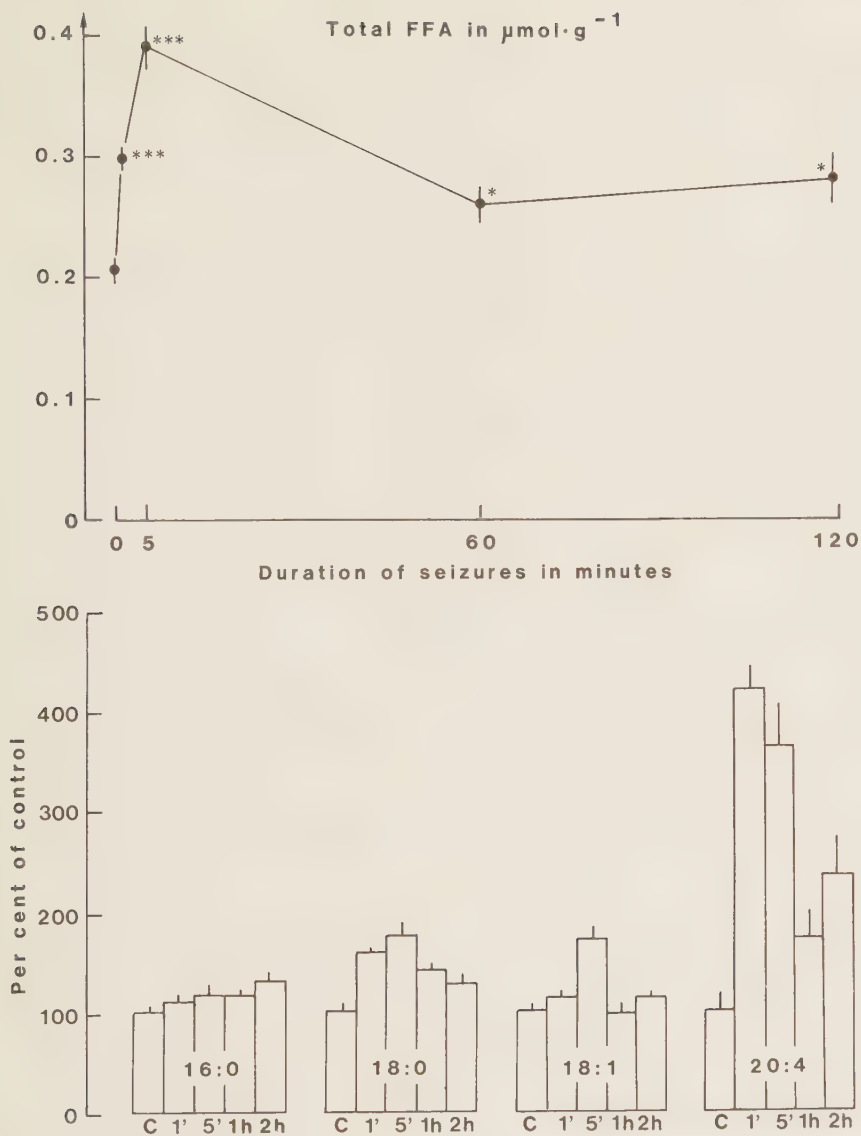


Fig.2. Time-dependent changes in cerebral cortical concentrations of a) total FFA content (upper fig.) and, b) individual FFAs (lower fig.) during continuous pentylenetetrazol-induced seizures of 1-120 min duration.

	Control n = 6	20 min n = 6	60 min n = 6	120 min n = 6
Frontal cortex	1.64 $\pm$ 0.16	2.49 $\pm$ 0.35	2.48 $\pm$ 0.17**	1.68 $\pm$ 0.23
Caudoputamen	1.38 $\pm$ 0.11	1.68 $\pm$ 0.14	1.96 $\pm$ 0.17*	1.82 $\pm$ 0.29
Globus pallidus	0.62 $\pm$ 0.07	2.53 $\pm$ 0.31***	2.56 $\pm$ 0.10***	2.24 $\pm$ 0.22***
Subst.nigra	1.09 $\pm$ 0.05	3.65 $\pm$ 0.38***	3.48 $\pm$ 0.15***	2.73 $\pm$ 0.36**
Nucleus ruber	1.43 $\pm$ 0.06	4.25 $\pm$ 0.43***	4.49 $\pm$ 0.17***	4.58 $\pm$ 0.33***
Nucleus accumbens	1.46 $\pm$ 0.14	3.22 $\pm$ 0.53**	3.74 $\pm$ 0.35***	3.23 $\pm$ 0.42**
Cerebellum	0.87 $\pm$ 0.05	2.29 $\pm$ 0.37**	2.84 $\pm$ 0.28***	2.65 $\pm$ 0.32***
Sens.mot.cortex	2.01 $\pm$ 0.24	4.44 $\pm$ 0.58**	4.23 $\pm$ 0.35***	3.74 $\pm$ 0.40**
Parietal cortex	1.69 $\pm$ 0.21	3.92 $\pm$ 0.46**	3.63 $\pm$ 0.24***	3.26 $\pm$ 0.36**
Thalamus	1.24 $\pm$ 0.08	5.14 $\pm$ 0.73***	5.32 $\pm$ 0.38***	3.98 $\pm$ 0.56***
Lat.thal.	1.10 $\pm$ 0.07	4.85 $\pm$ 0.64***	5.22 $\pm$ 0.53***	4.99 $\pm$ 0.87**
Visual cortex	1.20 $\pm$ 0.09	3.23 $\pm$ 0.36***	3.58 $\pm$ 0.12***	2.98 $\pm$ 0.36***
Lat.genic.body	1.38 $\pm$ 0.09	3.89 $\pm$ 0.39***	4.06 $\pm$ 0.22***	2.90 $\pm$ 0.45**
Sup.colliculus	1.37 $\pm$ 0.05	4.29 $\pm$ 0.37***	4.66 $\pm$ 0.27***	4.37 $\pm$ 0.41***
Auditory cortex	2.43 $\pm$ 0.13	4.17 $\pm$ 0.50**	4.01 $\pm$ 0.18***	3.66 $\pm$ 0.34**
Med.genic.body	1.94 $\pm$ 0.09	5.33 $\pm$ 0.59***	5.30 $\pm$ 0.35***	4.55 $\pm$ 0.53***
Inf.colliculus	2.13 $\pm$ 0.09	4.85 $\pm$ 0.34***	6.71 $\pm$ 0.36***	4.82 $\pm$ 0.45***
Temporal cortex	1.02 $\pm$ 0.04	2.31 $\pm$ 0.26***	2.47 $\pm$ 0.19***	2.27 $\pm$ 0.20***
Cingulate cortex	1.28 $\pm$ 0.12	4.79 $\pm$ 0.57***	5.16 $\pm$ 0.27***	3.81 $\pm$ 0.52**
Hippocampus	0.84 $\pm$ 0.08	2.04 $\pm$ 0.26***	2.11 $\pm$ 0.15***	2.12 $\pm$ 0.18***
Amygdala	0.98 $\pm$ 0.10	2.98 $\pm$ 0.35***	3.02 $\pm$ 0.19***	2.98 $\pm$ 0.23***
Septal nucleus	0.89 $\pm$ 0.09	2.71 $\pm$ 0.26***	2.71 $\pm$ 0.23***	2.45 $\pm$ 0.24***
Habenula	1.60 $\pm$ 0.13	4.32 $\pm$ 0.46***	4.58 $\pm$ 0.36***	4.11 $\pm$ 0.41***
Mamillary	1.84 $\pm$ 0.22	5.31 $\pm$ 0.74***	5.99 $\pm$ 0.42***	5.20 $\pm$ 0.37***
Hypothalamus	0.96 $\pm$ 0.06	2.75 $\pm$ 0.31***	3.08 $\pm$ 0.16***	2.83 $\pm$ 0.15***

Table 3 Local cerebral blood flow in rats subjected to pentylenetetrazol induced seizures.

1976). In brains of animals with 2h of seizure activity the brains looked macroscopically normal and firmly fixed. Light microscopically the cortex and the hippocampus showed marked changes of mainly two types, i.e. nerve cell changes and status spongiosus. The cerebellum, including the Purkinje cell layer, looked light microscopically normal.

a. Nerve cell changes In the cortex the nerve cell changes were of the same type as earlier described in bicuculline-induced status epilepticus (Söderfeldt et al. 1981) and could therefore be classified in the same categories. About half of the cortical neurons were in LM characterized by a change in staining quality, resulting in darker than normal neurons, often with triangular shape and shrunken cytoplasm, surrounded by perineuronal vacuoles (type 1) (Fig.3). In most of these abnormal neurons the nucleus could be discriminated from the cytoplasm (type 1a), but in about one fifth the internal structures could not be discriminated from each other (type 1b). The type 1 cells were most common in cortical layer 3.

In EM the type 1a injured neurons showed increased electron density of the nucleus and cytoplasm (Fig.4). The mitochondria looked either normal or showed loss of their inner matrix. The type 1b injured neurons (Fig.5) had very electron dense cytoplasm with multiple groups of vacuoles. Most probably, these vacuoles represented dilated Golgi cisternae. In addition many mitochondria appeared swollen which change seemed to be more frequent than in bicuculline-induced epilepsy.

In the deeper cortical layers another type of injured neurons was found. About one tenth of the cortical neurons had this type of alteration. These neurons (type 2) had normal staining characteristics but had slit-formed perinuclear cytoplasmic vacuoles (Fig.6). EM showed that most vacuoles represented widened portions of the rough endoplasmic reticulum, including the nuclear envelope. The type 2 injured neurons also contained small vacuoles, apparently dilated Golgi cisternae. Most of the mitochondria looked normal.

In the hippocampus LM showed that many neurons had configurational and staining abnormalities, resembling the type 1a injured neurons in the cortex.

b. Status spongiosus. The neuropil showed generalized sponginess in cortical layer 3 and, to a lesser and more variable extent, in layers

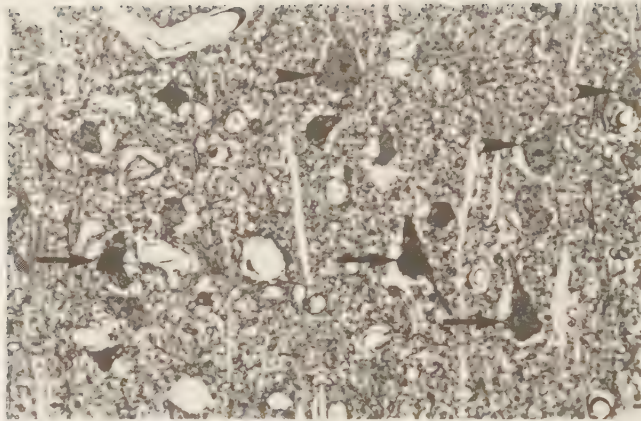


Fig.3. Cerebral cortex, layer 3 after 2h of status epilepticus. The tissue is markedly edematous. Type Ia (arrowhead) and Ib (arrow) injured neurons are surrounded by perineuronal vacuoles. Epon and toluidin blue X500.

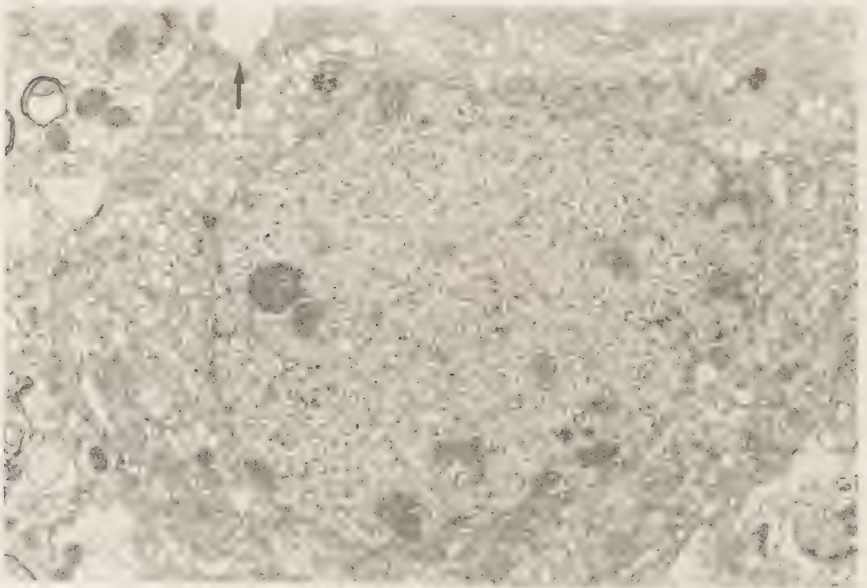


Fig.4. The incipient type Ia injured neuron is slightly condensed with minor edema of the perivascular astrocytic processes. The neuronal cytoplasm contains small vacuoles of both Golgi and ER origin. Most mitochondria appear quite normal, except for one (arrow) with marked swelling. X14000.

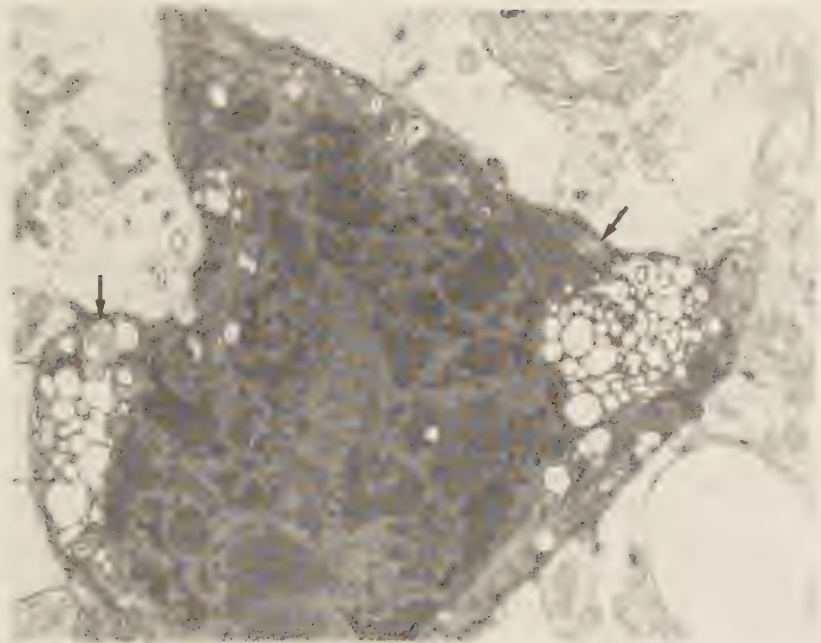


Fig.5. In a type 1b injured neuron both the nucleus and cytoplasm are condensed to such an extent that it is difficult to distinguish them from each other. There are two clusters of small vacuoles, apparently of Golgi origin. Mitochondria are somewhat swollen (two marked with arrows). Perineuronal astrocytic processes are markedly edematous. X15000.

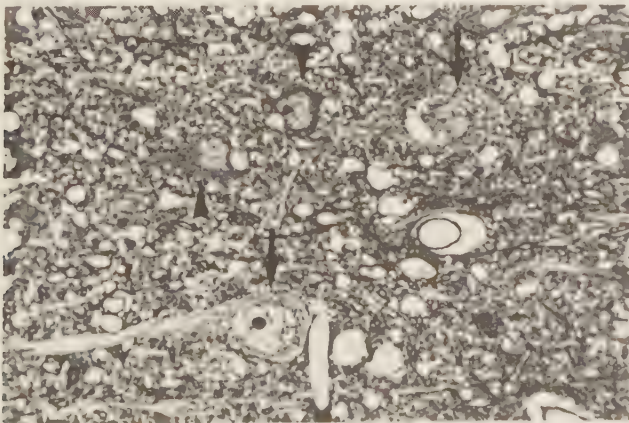


Fig.6. Cerebral cortex, layer 5 after 2h of status epilepticus. Both neurons with type 2 injury (arrow) and type 1a injury (arrowhead) are seen. Epon and toluidin blue. X500.

5 to 6. Remarkable, the outermost layers retained their compactness. In the hippocampus a pronounced edema was found in area CA 1 to CA 4. Also in the dentate gyrus edema was present, albeit less pronounced. EM on the cerebral cortex showed that this sponginess results from swelling of glial processes. Often these processes had a perivascular position or were seen close to injured neurons of type 1a or 1b. A close interrelationship between the shrinkage of the neurons (type 1 injury) and sponginess of the neuropil was observed. Many astrocytic cell bodies were swollen, also in areas without marked sponginess of the neuropil. The nuclear chromation in most astrocytes remained evenly dispersed.

The extent of the damage appeared comparable to that seen during bicuculline-induced status epilepticus. There was some interanimal variation; the least severe injury corresponding lto that seen after 1h of bicuculline-induced seizures (50 per cent injured neurons) and the more severe being comparable to that after 2 h of bicuculline seizures (65 per cent injured neurons).

DISCUSSION

As stated in the introduction, elucidation of the pathogenesis of epileptic cell damage required that complicating systemic events can be avoided and that cerebral metabolic and circulatory correlates can be assessed. Furthermore, since shortlasting seizures,(even if repeated), do not seem to elicit irreversible cell damage (Brennan et al. 1972; Mouritzen-Dam et al. 1980) it is also required that the seizures can be maintained for some time before the tissue is fixed by perfusion for assessment of any structural alterations incurred.

As judged by results obtained on baboons (Meldrum et al. 1973) and on rats (Blennow et al. 1978, Söderfeldt et al. 1981, Attila et al. 1983) bicuculline-induced seizures of at least 60 min duration give histopathological signs of cellular injury. However, it is presently a matter of speculation whether changes observed at the end of a sustained seizure period represent reversible or irreversible events. Thus Brown et al. (1980), working on ventilated cats, failed to find that bicuculline-induced seizures of 5 hrs duration induced cell damage in hippocampal (or cortical) pyramidal cells when seizures were arrested for 1 to 5 hrs prior to perfusion fixation of the tissue.

Furthermore, in the experiments of Purpura and Gonzales-Montegudo (1960) seizures of 2-6 hrs duration were accompanied by hippocampal neuronal damage only when the seizures were induced by methoxyppyridoxine, but not by strychnine or pentylenetetrazol. Finally, our own studies showed that the number of cells with structural alterations, conventionally assumed to represent overt cell damage, were drastically reduced if a recovery period of 2 h preceded perfusion fixation (Söderfeldt et al. 1983, Attila et al. 1983). Nonetheless, a small proportion of the neurons appeared to have sustained irreversible damage.

At first sight, induction of seizures by administration of bicuculline to ventilated and well oxygenated animals, with maintenance of arterial blood pressure, appears to provide a model which excludes systemic complications as contributing pathogenetic factors. However, the model suffers from three drawbacks : a massive sympathoadrenal activation at seizure onset gives an abrupt increase in arterial blood pressure which is difficult to abort completely, a very pronounced plasma acidosis develops, and the seizures are difficult to arrest. Two pertinent questions arise. First, are the structural alterations observed secondary to the systemic alterations, e.g. to microcirculatory changes elicited by such alterations? Second, are the structural alterations only observed when bicuculline is used as seizure-inducing agent, or are they characteristic of prolonged seizures in general?

It was with these questions in mind that we decided to study electroencephalographic, metabolic, circulatory, and structural alterations during seizures induced by another drug which, although assumed to act as a GABA blocker, seems to induce dysinhibition by a different mechanism than that of bicuculline (Marangor et al. 1979; Simmons 1980). Our first objective was to examine the characteristics of the EEG discharges and to characterize the systemic alterations induced. Our second objective was to explore the cerebral metabolic and circulatory changes which may correlate to the development of structural alterations, mainly the perturbation of cerebral energy state, the extent of the lactic acidosis, and the characteristics of the accumulation of potentially toxic FFAs (see Siesjö 1981).

As the results showed, pentylenetetrazol induced EEG changes indistinguishable from these previously recorded during bicuculline-

induced seizures. Also the systemic alterations were similar in that seizure onset was accompanied by an abrupt blood pressure peak, and that a pronounced plasma acidosis developed (cf. Plum et al. 1968, Howse et al. 1974). In general, cerebral metabolic changes, recorded in the present study, were very similar to those previously measured in bicuculline-induced seizures (Chapman et al. 1977, Blennow et al. 1979, Folbergrova et al. 1980, Siesjö et al. 1982), as well as to those measured during shortlasting seizures elicited by pentylenetetrazol-seizures (Duffy et al. 1975, Ferrendelli and Kinscherf 1977). Essentially, these changes consist of an initial moderate perturbation of cerebral energy state, by a reduction in tissue glucose and glycogen concentrations with elevated lactate and pyruvate concentrations (and lactate/pyruvate ratios), and by a transient increase in cAMP and a sustained increase in cGMP concentrations. As during bicuculline-induced seizures (Folbergrova et al. 1981), these alterations were less pronounced in the cerebellum whose metabolic state was clearly perturbed only initially. We also note that, as during bicuculline-induced seizures (Siesjö et al. 1982) increases occurred in cerebral cortical FFA concentrations, the largest relative changes recurring in the urachidonic acid concentration. On the whole, therefore, systemic and metabolic changes accompanying pentylenetetrazol-induced seizures appear similar to these provoked by bicuculline.

Since the present experiments were carried out almost 2 years later than those describing CBF changes during bicuculline-induced seizures (Ingvar and Siesjö 1982), and since interstrain variability cannot be excluded, a direct comparison is difficult. Nonetheless, it is tempting to conclude that the cerebral vascular responses differ. Thus, in the present experiments marked hyperemia affected a larger number of structures, and we could find no signs of a secondary deterioration of CBF in the period 60 to 120 min (cf. results reported by Ingvar and Siesjö 1982). It remains to be shown if a difference in vascular response exists between the two models and, if so, what constitutes the mechanisms responsible.

We found that the structural alterations, as studied at the end of a 2 h period of sustained pentylenetetrazol-induced seizures, did not substantially differ in quality or extent from those found after a comparable period of bicuculline-induced seizures (see Söderfeldt et al. 1981). Thus, both the type 1 a and b, and the type 2 changes were

present, and their localization was as previously described. Furthermore, a rough estimation indicated that the extent of damage was similar, as was the extent and localization of status spongiosus. Finally, the ultrastructural characteristics of the type 1 and 2 changes were similar except for the apparently more frequent swelling of mitochondria.

In conclusion, the present results provide the following answers to the three questions posed in the beginning of the discussion. First, the structural alterations induced during bicuculline-induced seizures are not peculiar to that drug, but also occur during seizures induced by another drug, assumed to cause dysinhibition by interacting with GABA-ergic mechanisms at another site. Second, the accompanying systemic alterations and cerebral metabolic changes are similar in the two models but, possibly, cerebral circulation is better maintained during pentylenetetrazol-induced seizures. Third, with neither of these models can systemic alterations be entirely prevented and it cannot be excluded that these alterations contribute to any permanent damage incurred. In order to explore if such damage occurs, recovery experiments are highly warranted. In this respect, pentylenetetrazol-induced seizures offer some potential advantages since cerebral microcirculatory problems seem minimal.

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